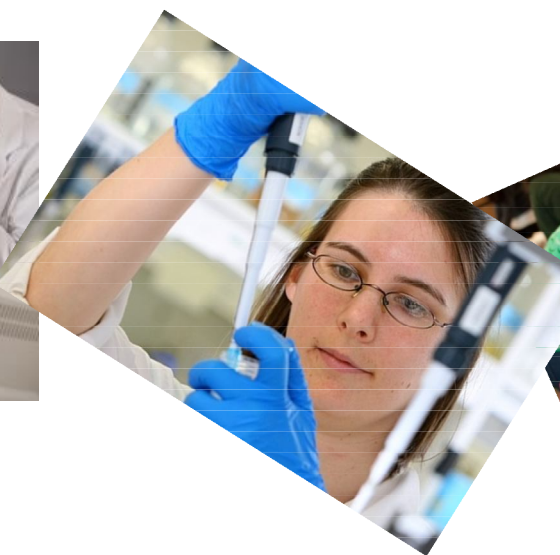




An Epidemiologic Review of

Mycobacterium tuberculosis

An epidemiological and historic review of Mycobacterium tuberculosis, that elaborate the microbiological, pharmacological and molecular features of tuberculosis.

Dr. Taha Nazir

B.Pharm., M.Phil. (Pharmacology)

Ph.D. (Microbiology)

Scientific Executive (Infection Control & Research),
[ICDTD Inc.](http://www.icdti.org), 17-937 Northumberland Ave Saskatoon
SK S7L3W8 Canada.

E.: taha@icdti.ca; W.: <http://www.icdti.org>

An Epidemiological review of **Mycobacterium tuberculosis**

1st Edition (2015)

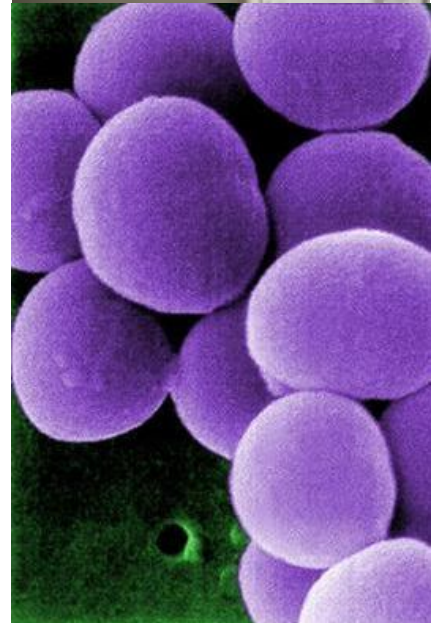
Compiled by:

Dr. Taha Nazir

B.Pharm.(PU), R.Ph., MBA,
M.Phil. (Pharmacology), Ph.D. (Microbiology).

E.: taha@icdttdi.ca hoo.com;

Skype: dr.tahanazir; Viber: Taha Nazir;



NOTICE

The therapy plans and clinical profiles of therapeutical agents are being changed every day. Pharmaceutical research and clinical trails have sharply broadened our knowledge and making more sophisticated alterations in treatment protocols. The author, contributors and publisher of this book have checked the informations with sources believed to be reliable in their efforts to provide information that is complete and generally in accord with the standard accepted at the time of publication. However, in view of the possibility of human error or change in latest clinical research data, neither the author nor the publisher nor any other party who has been involved in the preparation or publication of this work warrants that the information contain herein is in every respect accurate or compete, and they disclaim all responsibility for any error or omissions or for the results obtained from use of the information contained in this work. The students, researchers and readers are encouraged to confirm the information contains

PREFACE

The microbial infectious diseases demand an integrated and valid collection of simple and conceptual informations, which support the health professional in clinical practice. Therefore, "An Epidemiological Review of Mycobacterium tuberculosis" describes comprehensively the epidemiology, pharmacology and microbiology of Mycobacterium tuberculosis. We also have tried to cover the history, general features and molecular characteristics of Mycobacterium. Thus, we have carefully tried to present the latest and authenticated informations that may potentially help to improve the patient's care and minimize the complications of polypharmacy.

Additionally, the poor pharmaceutical care and substandard clinical facilities are major factors to introduce the resistance against first line antituberculosis drugs. That seriously needs special attention to save the innocent lives (especially in developing parts of the world). There are so many avoidable casualties that can be saved by offering correct medication on behalf of accurate differential microbiological diagnosis. But; unluckily, the patients passed away because of this terrible monstrous health care system monster disease. The inappropriate microbiological, clinical, and pharmaceutical care works like an invisible vampire. Therefore, the clinical microbiologists, immunologists and pharmacists have to play their role to enforce, implementation and monitoring the standard facilities. We also need to develop the scientific skills and collaborative relationship to produce integrated professional workplaces. That structured learning process will then help to improve the overall clinical outcomes. The "An Epidemiological Review of Mycobacterium tuberculosis" is therefore compiled to present the required learning's. The material contained herein facilitates an in-depth level of understanding of essential knowledge of *tuberculosis*.

The valuable feedback and constructive suggestions will warmly be welcomed

Dr. TAHA NAZIR

B.Pharm.(PU), R.Ph., MBA, BA.
M.Phil. (Pharmacology), Ph.D. (Microbiology).
E.: tahar@icdttdi.ca

October 21, 2015

CONTENT

Chapter No.	Title
Chapter 1.	Historic profile of tuberculosis
Chapter 2.	Epidemiological review of tuberculosis
Chapter 3.	General and microbiological features of mycobacterium
Chapter 4.	Molecular features of mycobacterium tuberculosis
Chapter 5.	Pathogenesis of tuberculosis
Chapter 6.	Resistance of mycobacterium tuberculosis
Chapter 7.	Molecular basis of resistance
Chapter 8.	Diagnosis and prognosis
Chapter 9.	Treatment of tuberculosis
Chapter 10.	Prevention of tuberculosis

Chapter 1.

HISTORIC PROFILE OF TUBERCULOSIS

Phthisis – Greek word indicating the character of “wasting away”, scrofula – swelling of the lymph nodes of the neck, white plague – Tb epidemic term introduced in Europe during the 18th century and tuberculosis – the presence or product of tubercle bacilli are all words used for tuberculosis, making specific points in history. This invisible enemy continues to challenge man’s knowledge and mock his efforts. The “captain of the men of death” continues to march forth leaving behind a trail of human misery, economic chaos and death. Since antiquity *tuberculosis* presents in human population. The spinal column fragments from Egyptian mummies 2400 BCE show explicit evidence of this disease. In 2000BC it was subject of hymn in an Indian sacred text. There were also some reference of lung scarring in preserved bodies (mummies) resemble to modern tuberculosis drive toward that tuberculosis was exist among the Americans since 2000 BCE. The term “phthisis” meaning consumption appears first in Greek literature. Around 460 BCE Hippocrates identified phthisis as the most widespread disease of the time and noted that it was almost always fatal. During 2500 -1500 BC TB was recognized in main Europe countries and made the earliest confirmation of the tuberculosis. While; in Britain it came from grave dating of 55BC- 800 AD through the roman occupation. Thus; in mid 17th century in London, one in five deaths; as recorded in mortality bills, were because of the consumption (the term used for tuberculosis at that time). Tuberculosis was also known as “white plague” in Europe because of massive death and whitish skin coloration of patients. Recently; the DNA of *Mycobacterium tuberculosis* identified in pulmonary lesion of 1000 year old human remains in Peru. Thus; the exact anatomical and pathological depiction of tuberculosis started from identifying the characteristic modifications and appearance of actual tubercle in lungs or other body parts of patients. The development to abscesses and cavities also were described. Tuberculosis was also more commonly referred as vampire during the era of industrial revolution; when one infected member of family lost his health while other passed away because of this disease. Furthermore tuberculosis patient’s exhibited red swollen eyes; coughing blood and pale skin were supposed be the need of replenishment. This may be the medication of the common mythology of the tuberculosis vampire origination. In 1865 Jean Anthonio demonstrated the transmission of TB by inoculating the sputum of patient to rabbit. The bacillus causing TB was identified and described on 24th March, 1882 in monthly meeting of Berlin physiological society by Robert Koch. He also isolated the tubercle bacillus. Same year Paul Ehrlich discovered the staining method of mycobacterium tuberculosis. Robert Koch was awarded the Nobel Prize in 1905 (physiology and medicines) for his discovery. 25th March celebrated every year to commemorate this glorious achievement. By the turn of 19th century, there as seven million estimation of worldwide TB death rate per year along with 50 million per year pulmonary TB rate. This may lead to the dreadful situation of destruction of European civilization by the end of the 19th century. While; in 1939, Schonelein gave the name “tuberculosis” to this disease. In 1942, two American scientists Dr. Hinshaw and Feldman reported the 1st remedial medicine trailed (Promin) in tuberculosis treatment. But unluckily; this trial was failed because of sever adverse effects. Dr. Schatz, Bugie and Waksman; in January 1944 to declared to the world streptomycin discovery. A 21 year old tuberculosis patient girl ‘Patricia’ was treated successfully with this medicine. Dr. Lehmann in 1946 discovered para-amino-salicylic acid (PAS) for treatment of tuberculosis. In 1952, a new wondered medicine “isoniazid” was published in paper by Dr. Robizek and Selikoff. It was presented in New York to use against tuberculosis by Dr. Robizek and Selikoff. In France; the vaccine BCG (bacillus calmette guirine) was introduced following a 50,000 children survey that indicated 80% decline in infant rate. Dr john Crofton an expert of tuberculosis at University of Edinburgh has proposed a combination of drug, PAS, streptomycin and isoniazid to completely cure the tuberculosis in 1960. Then; he announced “all out war” to defeat the tuberculosis. In the USA; a survey was

conducted (1961- 68) to investigate susceptibility of drugs among hospitalized tuberculosis patients. That showed 3.5% resistance against anti-tuberculosis drugs in 1970. WHO affirmed TB as global emergency in 1993. That causes more morbidity and motility than any curable infectious disease. In 1993-94 WHO was recommend the DOTS – Directly Observed Treatment Strategy. In 1997 International Union Against Tuberculosis & Lung Diseases (IUATLD) and WHO launched DRS (Anti-tubercular Drug Resistance Surveillance) a worldwide plan. Thus; WHO and several partners conceived the DOTS Plus and working group for multidrug resistant tuberculosis.

Chapter 2.

EPIDEMIOLOGICAL REVIEW OF TUBERCULOSIS

WHO estimated that one third of world's population; about 1.7 billion people are carrier of tubercle bacillus and 8 million new patients of tuberculosis causing 3 million deaths to every year (World Health Organization, 2004). WHO projects that, by the year 2000 AD, the annual figures instead of going down will further grow up to ≥ 3 million new tuberculosis patients along with 3.7 million casualties (Raviglione M.C, 1995). Thus; the problem is more aggravated by pandemic of increased incidence of multidrug resistant tuberculosis and AIDS (Herendra & Shah et al., 1998)

World Wide Prevalence Tuberculosis

Tuberculosis is the main cause of death. That caused about 3 million deaths every year) (Bloom BR & Murray, 1992). There are about 8.8 million new TB patients are enrolled in 2003 and 80% of these patient belong to only twenty two countries of world. More than 80% of tuberculosis patients belong to the most important age group of 15 - 49 years. Thus; approximately 252,000 tuberculosis patients passed away every weekly that make $\geq 7,000$ each day. However; the death rates is big threat for mankind in this only glob. It is curable but leading killer among HIV infected people. $\frac{1}{4}$ of a million tuberculosis casualties belong to the HIV and majorly from the African countries. Tuberculosis is the leading cause of mortalities in young women especially in Africa. If tuberculosis is left unrestricted, it may kill further thirty five million people within next twenty 20 years. Thus; tuberculosis is still growing at the rate of 1% yearly due to fast increase in Africa. One in ten tuberculosis bacilli carrier people are infected with this mortal disease. According to a recent WHO and partner survey MDR tuberculosis exist in almost all (109) countries of the world. Approximately 10425,000 New MDR-tuberculosis patients produced every year with the highest rate in china and Ex USSR; where about 14% of all new patient are MDR (WHO, 1997) The deprived socioeconomic structure and emergence of AIDS has major contribution in diseases resurgence especially in most industrialized area of the world (Barnes P, 1991). While; in developing countries, the disease has always been widespread. Moreover; the global HIV has increased its ruthlessness. There are rapid pandemic and extensive social restructuring noticed due to rapid industrialization. Thus; tuberculosis becomes a major worldwide public health problem. It seems like an emergency in glob (Ashok Rattan, 1998). Moreover; tuberculosis is the leading cause of mortality in adults and make a 26 % of all avoidable adult demises worldwide. World Health Organization reported 8 million tuberculosis (TB) patients each year, resulting in 3 million casualties (Raviglione & Sinder, 1995). The resistances of Mycobacterium tuberculosis against Antituberculosis drugs have been recovered from both immunocompromised and immunocompetent patients (Cohn & Bustreo, 1997)

The new advancements in health care facilities, progressive policies and rational treatment protocols particularly in developed part of the world has offered the goals to eliminate tuberculosis by the end of the 20th century. But it is reemerged because of the epidemic of global human immunodeficiency virus (HIV). Additionally; the tuberculosis control programs partially disrupted because of the entrapment of huge population in poverty. However, 95% of tuberculosis patients are seen in developing countries (Pablos-Méndez et al. 1998). Thus tuberculosis has remained endemic for many decades (Maria Ines Moura Friexo, 2004). It kills a big population in their most productive young age. That contribute an economically deficit in society. Approximately 9 million of new tuberculosis patients produced every year. While; 2 million active patients died; that makes it the biggest killer after HIV.

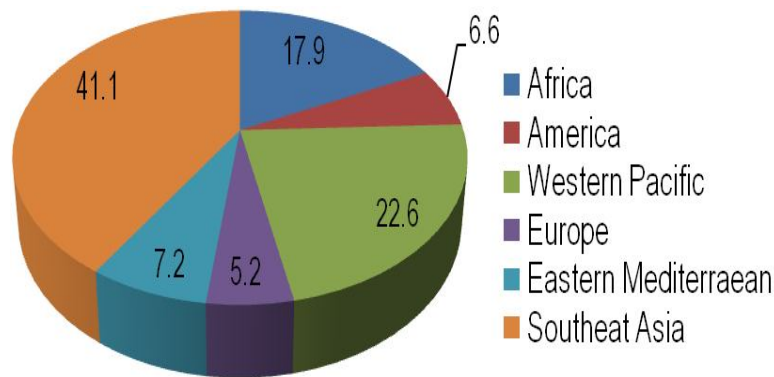


Figure 1. Global incidence of tuberculosis, the estimated 8.8 million cases worldwide, more than 40% of the cases are in Southeast Asia; India has approximately 53.3% of those cases.

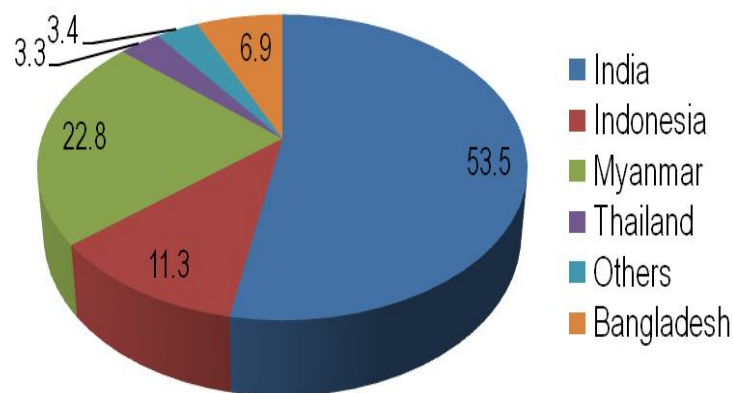


Figure . Prevalance of tuberculosis in South East Asia.

*Others include Bhutan, 0.05%; Nepal, 1.2%; Maldives, 0.001%; Sri Lanka, 1%; DPR Korea, 1.2%.

(Ashok Rattan et al., 1998)

Thus; the poorly implemented DOTS provides an increase in resistance of tubercle bacilli. The pervasiveness of resistant *Mycobacteria* demands treatment through DOTS plus program. There are two major problems faced in Tuberculosis control programmes; HIV and drug resistance. 2.5 million HIV patients out of the 6 million people are infected with tuberculosis. Approximately 60% of AIDS patients develop tuberculosis indicating the most frequent life threatening opportunistic infection associated with HIV (WHO, 2004)

Prevalence in South East Asia

Approximately 38% of the world tuberculosis burden is in the South East Asian countries. Where 750 000 patient died annually because of tuberculosis.that makes 1500 every day, or 1 after every minute. Five out of the 22 highest burden countries are in South East Asian region (WHO, 2004).

Transmission of *M. Tuberculosis*

The risk for an individual of becoming infected with tubercle bacilli depends on the concentration of organisms in the source case, the duration of exposure to air contaminated with tubercle bacilli and the aerodynamics of the droplet nuclei: Patients with infectious tuberculosis may have between 10^7 and 10^8 organisms in a cavitating lesion. A 10μ droplet nucleus may carry three to ten tubercle bacilli. In indoor

environments, droplet nuclei can remain suspended in the air for long periods of time, unless they are removed by ventilation or filtration. Virtually all transmission occurs in enclosed environments. The infective dose is very low and may constitute fewer than 10 tubercle bacilli, only about 6% of inhaled organisms reach the lung alveoli; the majority of inhaled particles settle in the upper respiratory track and are expelled or harmlessly swallowed and digested. The probability of a person becoming infected during a one hour exposure period has been estimated to range from 1 in 600 (0.2%) to 1 in 4 (25%). Contaminated clothing, bedding, eating utensils and books etc. are not involved in the spread of tuberculosis infection and need no special attention. Infection rarely occurs when bacilli are introduced through the skin. This is occasionally seen among pathologists and laboratory workers handling infected specimens. Human sputum is, however by far, the most important source of infection and other infected body fluids present no practical risk to the majority of HCWs. Contact therefore does not mean infection (C. Karin Weyer et al., 1997). Thus; the contacts person should be investigated for resistant because of having exposure of patient with Multi drug resistant tuberculosis. That may get infected and should be examined to assess the likelihood of the actual infection. That might be a multi resistant strain of *Mycobacterium tuberculosis*. The considerable factors that may contribute are as under;

- 1 The probability of exposure of persons with drug susceptible/ resistant tuberculosis patient.
- 2 Closeness type and intensity with source infectious patient.
- 3 Duration of sharing the air space with tuberculosis patient and type of sharing e.g. household member, hospital roommate etc that possess more risk for infection than those with a short exposure.
- 4 The closeness and intensity of the exposure;
- 5 The pattern and level of resistance of source infectious MDR patient.
- 6 Exposure in an enclosed, small and poorly ventilated space is more likely to transmit this bacillus as compared with an open, large and well ventilated space. Thus; in conclusion, the exposure during cough inducing procedures e.g. bronchoscopy, sputum induction etc may seriously increase transmission (Karin et al., 1997)

Nosocomial or hospital acquired infection

Implicit and confirmed community and nosocomial exposure can be investigated by reviewing the medical record of the treatment and residential facilities. Apparently; the nosocomial transmission was considered if the newly identified patient having the same section of the institution. Usually symptoms of the tuberculosis are grown up at least after 30 days of infection. While in most of the cases; disease arrest immunity multiplication and prevent clinical outcomes. The 20% - 50% of immunocompetent persons may get infected if exposed to tuberculosis for a long period (hours or days rather than minutes). While; among 90% of infected persons the *M. tuberculosis* may become dormant and will not produce the clinical symptoms of disease. 5% of person may develop an early stage of active tuberculosis (usually within two years after infection). Moreover; the further 5% individuals may develop tuberculosis at some point during their resting life time. Mostly the suppression of immune system because of any physical or emotional stress may contribute to get infection with this disease (Karin et al., 1997)

Community transmission

The community transmission of tuberculosis is considered if any of the following has taken place:

- 1 The patient exposed to another patient having same strain of *Mycobacterium tuberculosis* and was infectious at least thirty days before illness beginning in the following patient. The exposure may occur in single room occupancy hotel at home, homeless shelter or other non-institutional surroundings.
- 2 The patient contacts another patient, whose *Mycobacterium tuberculosis* has the identical DNA pattern or multi drug resistance profile, but DNA genotyping test results are not obtainable.

The patients should have an epidemiologic link, if investigation of community or nosocomial transmission was confirmed. While; the source patient is not be considered to confirm the epidemiologic link. The computerized outpatient clinic records and information of hospital ward, bed and floor can be used to analyze the spatial and temporal overlap of cases. The Medical records can be reviewed for patients who breach the isolation protocol during their hospitalization. Moreover; the additional demographic and social information may be collected through questionnaires. Particularly, the patients were investigated for where and with whom they spent significant time along with the additional personal and background information. The infected patients may also be asked about how and where they think they get exposed for tuberculosis (Sonal et al., 1997). The adult's pulmonary tuberculosis are the main infectious source of M. tuberculosis that may also contribute the multi drug resistant tuberculosis. The droplets or particles of infective patient are generally resulting from moist forced expiration through the mouth discharged into the air. Mostly the sneezing, spitting, coughing or by procedures of liberating aerosols from nose or throat eventually produce other tubercular patients. Approximately 1-5 microns in size, the infective particles are produced if an aerosolised material dried out to form droplet nuclei in lungs. The trapped and inhaled resident alveolar macrophages droplet nuclei remained airborne to initiate the lung infection (Karin et al. 1997).

Molecular Epidemiology

The extensive transmission of multi drug resistant M. Tuberculosis strains take place during the outbreak of 1980s and in beginning of 1990s. The epidemic was identified in many hospitals. The subsequently these outbreaks were associated with one strain known as the "W" strain of tuberculosis. While; in many of the strains were resistant to rifampicin, isoniazid, streptomycin and ethambutol. Though, other multi drug resistant strains were linked with epidemic and transmission during that time. A survey of molecular epidemiology conducted in New York City indicated that multi drug resistant tuberculosis was linked with clustered strains of *Mycobacterium tuberculosis*. That suggests a current transmission of the bacilli. Since 1992; when an enhanced Tuberculosis Control Program was implemented, the incidence of susceptible and resistant tuberculosis has been reduced rapidly. By 1994; 21.5% of the tuberculosis patients are decreased. While; the 60% of MDR tuberculosis cases are reduced. In 1995; Tuberculosis Control Program was started by DNA genotyping of new MDR tuberculosis strains to understand betterly the epidemiology of MDR tuberculosis in New York City. The main objective of this study was to provide the descriptive molecular epidemiology of MDR tuberculosis in during 1995 – 1997. That may also help to recognize major MDR strains exist during that time (Sonal et al., 1997).

Risk Factors Contributing the Transmissions of TB

Short term therapy is the backbone of pharmacotherapy of tuberculosis (Kochi, 1993). The patient compliance and rational prescriptions always assure the treatment. In fact, tuberculosis incidences were progressively decreasing in most of the industrialized part of the world; until the trend was reversed (Bell RT.1992). The significantly contributing or associated factors are as under; lower education, female sex, more than six instances of health seeking encounters, visiting a private doctor/ traditional healer and

outpatient diagnosis. Ladies are most likely; more vulnerable and susceptible because of fear of stigma, combined mobility, additional workload and limited financial resources. Thus; in nutshell; the financial burden produce barriers in prompt diagnosis particularly among women. Significant financial obstacles including cost of 'special food', transportation expenditure and lost income are main hurdles in successful eradication of tuberculosis. These obstacles can be reduced by certain interventions including the decreasing the travelling distance, duration of illness before diagnosis and number of health visits. Additionally, 58% of tuberculosis mothers disclosed the inability to feed their kids, get proper education and fulfil the daily needs of their life. The parental disease affects the continuation of 11% school children; 8% rural, 13% urban. Moreover; 8% of the school children join employment to support their families. While; 34% of patients, parents are not able to buy enough clothing or food or books for their kids because of the heavy expenditure spent on tuberculosis treatment. Thus; the mothers with tuberculosis affect the total family circle especially the children. The family unit disrupted because of loss of earnings and reduced routine family leisure activities. The socioeconomic effect of tuberculosis over individual families is an important factor to decrease the earning competence. Thus; the risks of families' disturbance increased in society. That's why; World Health Organisation has introduced the DOTS program to improve positively the overall well being of the family by reinstating the working ability through treatment protocols and reducing the wastage of revenue. Food consumption, resources and assets can be preserved considerably by implementing the DOTS program. That renders the family financial and physical resources. The likelihood of death of the sick factory laborer and rickshaw puller may be reduced significantly by improving the family welfare over the long run (Emanuele & Narain, 2002). The major contributing "epidemic" reasons to multiple drug resistance in certain parts of west are the outbreak of Human Immuno-deficiency Virus, overcrowding leading, homelessness and drug addiction. Multi drug resistance tuberculosis occurs either through drug resistant mutant's collection of innovative susceptible strain or by already patient with resistant *Mycobacterium tuberculosis*. Those are developed in result of inadequate therapy or poor patient adherence (acquired resistance) (Karin, 1997).

WHO Contribution (DOTS Programme)

World Health Organization has major contribution to control tuberculosis worldwide. Its monogram is mentioned here and services can be divided into following headings,

WHO Targets for TB: The two major targets to TB controls are,

- 1 World Health Assembly has targets in 2005 to identify at least 70% of tuberculosis infections cases (latest data 45%) and similarly provide treatment successfully to 85% of detected cases (82%).
- 2 While; the goals of millennium development has been targeted for 2015 by associated stop TB partnership; that aimed the reversal of tuberculosis incidence at the target of half of the prevalence and death by 2015 in comparison to 1990.

DOTS Strategy

Higher than 20 million tuberculosis cases are treated under DOTS program. While; 82 countries have taken up the DOTS strategy. Even though; a quarter of the world's population is still seeking access to DOTS program. The DOTS strategy was offered in 1995 consisting of 1) Government commitment to tuberculosis control. 2) Diagnosis through bacteriology and an effective laboratory network. 3) Standardized short term therapy with complete patient support. 4) Continuous supply of quality assured drugs. 5) Reporting and recording to measure the patient and programme outcome.

New global stop TB strategy

World Health Organisation has developed a new world wide stop tuberculosis strategy with aims of to reach all patients and strengthen the tuberculosis control activities. That have six key important components 1) Addressing tuberculosis, MDR-TB and HIV; 2) Pursuing the quality DOTS extension; 3) Contributing to strengthen the health care programs; 4) working groups; 5) Empowering the communities and patients; 6) Enabling and supporting the research activities. World Health Organisation stop tuberculosis department develops policies, standards to support the efforts and strategies in collaboration of the country offices and regional health care programs. The participant provinces and states measure the progress towards tuberculosis assesses and targets toward the national programmes performance, financing impact and partnerships facilities, advocacy and communication. While; the stop TB partnership secretariat is housed by WHO; that is an association of 400 stakeholders. That coordinates, bold and assign the responsibilities to all health care providers. They do advocate for communication social mobilization, DOTS plus, DOTS expansion, new drugs, MDR-TB, new vaccines new diagnostics, and tuberculosis HIV. The drug facility run by stop tuberculosis partner world wide is growing its access to drugs for DOTS; that has scaled up in just four years to provide 4 million successful tuberculosis treatments.

Green light committee

Green light committee work through DOTS plus project for access to quality Multi Drug Resistance tuberculosis at reduced cost in some cases by as much as 99%.

World wide TB strategy:

The blueprint recommendations for TB control, particularly in Africa during 2006-2007 are incorporated into development agendas to strengthen the DOTS programmes. Tuberculosis activities are expanded in partnership of HIV treatment. WHO declared tuberculosis as an emergency in Africa in 2005. While; the WHO's director for European region has warned the tuberculosis emergency in Europe. Even though; G8 world leaders offered to meet the needs to identify the stop TB partnership in 2005 in Africa. The development of new drugs and vaccines were encouraged in meeting of the financing needs of the world to fund in fighting against TB, AID and malaria.

Tuberculosis and prisoners

According to a susceptibility test a high occurrence of resistance to the 1st line tuberculosis drugs were experienced. There were only 21% patients had M. tuberculosis strains sensitive to all antibiotics, and 23% of patients with strains of multidrug resistance. Thus; overall, there was only 42% sputum conversion rate. Those are ranging 10 to 29% (MDR strains) to 61% (sensitive strains). The overall 54% treatment rate despite an excellent compliance. Certain operational limitations were noticed as under; 1). premature release or transfer of prisoners, 2). late identification of cases with consequent high death rate, 3). Convincing staff to directly observe patients while swallowing antituberculous medication or lack of proper inspiration, and 4) disease advancement. The existence of highly resistant tuberculosis strains in prisons is significant to affected prisoners; But also the hazardous for prison staff, their inmates, and for their families. Which should be considered especially in possible diffusion to the general population. While; the active tuberculosis patients in prisons can be controlled by early detection to improving outcome and decrease circulation. Antituberculosis therapy should be given to the patients when they are transferred or released; referred to DOTS centers or DOTS in all prisons (Emanuele and Narain, 2002).

Antitubercular organization

World Health Organization declared TB as a worldwide public health (Julio, 1997). The Center for Disease Control and Prevention National Surveillance Systems registered tuberculosis patients, including MDR-tuberculosis (Moore M. et al., 1997). There certain other local/ international, governmental/ non governmental anti-TB organization/ programmes launched are Pakistan Medical Research Council for Pulmonary & Chest Diseases (PMRC), National TB control programme, the NTP manager; National players, the National Drugs and Therapeutics Committee; the National TB Advisory Committee; the Procurement Office; the National Drug Regulatory Authority; the office responsible for inspection of stocks and distribution; the Legislative Office; the Financing Office and; the Pharmacovigilance Network.

Tuberculosis and AIDS

The out breakd and registration of smear positive tuberculosis patients are increased and most of these patients are sanctioned to the HIV pandemic. The relapse of tuberculosis or incidence of reappearance in a population with high level of coinfection with HIV observed intensively. Thus; being the most favorable period of tuberculosis management for individuals infected with HIV (human immunodeficiency virus) still undere discussion. The frequency of reversion in patients with HIV coinfectd was compared with HIV negative individuals. Tuberculosid reappearance was defined as having positive < 30 days culture after the very last theratpy date. While; the reversion having a positive culture at least 30 days after the last therapy. Thus; individuals HIV infected were more probable than non-infected to have recurrence or relapse. Individualsl HIV infected received more close to 36 weeks of therapy was more possible than those received higher than 36 weeks to have reappearance. In netshal., physicians, pharmacists and clinicians should be attentive of the likelihood of reappearance of tuberculosis in 6-9 months after the begining of therapy. Moreover; in compariable settings, sputum examination to make sure alleviation or appraisal of three months following completion of treatment should be done among individuals with HIV infection or have received the shorter regimen. The patients of HIV infection can be effectively cured with regimens of DOT anti-TB. Though, excess mortality of patients infected with HIV despite the successful treatment is different challenged faced in clinical practic. Therefoe; if it emerges significant to HIV infected patients treatment, then necessarily be further investigate to study the causes of high mortality (most probably becusue of opportunistic infections of other than HIV) to identify potential and successful interventions. (Emanuele and Narain, 2002). Presently; the human immunodeficiency virus is the most significant threat for disease development and following infection. Human Immunodeficiency Virus kills T4 lymphocytes or T-helper cells. That decreases the defense against infection of Mycobacterium tuberculosis. Therefore; Human Immunodeficiency Virus infection raised the danger of reactivation of inactive TB infection and the risk of progressive disease following new infection as well (Karin, et al., 1997).

World Wide Ideas

To highlight tuberculosis there are following main ideas used recognized the tuberculosis;

1. Education/ training.
2. Lobbing for funding.
3. Convincing/ discussion with thing tanks/ deciders Parades/ rallies.
4. Food & cloths for TB patient's.
5. Recognizing/ obliging the workers & partners.
6. Commemorative by postage stamps.
7. Poster competition.
8. Charity sports/ games.
9. New DOTS clinics

Chapter 3.

GENERAL AND MICROBIOLOGICAL FEATURES OF MYCOBACTERIUM

Mycobacterium has distinct general and molecular features. It can particularly be identified on basis of these specifications. It is non-motile, non-spore/ capsule forming, rod shaped & slow growing mycobacterium. It has Waxy mixture of lipid rich resistant cell wall, polysaccharides and remains dormant for decades. More than 50 species currently accepted by ICSB and 20 Species capable to cause human disease.

Species of M-TB

Forty one species of mycobacterium currently accepted by the International Committee on Systematic Bacteriology (ISSB). The species that are capable of causing human diseases are referred as obligate and opportunistic pathogens. In developing countries, the major pathogenic species are *M. leprae*, *M. tuberculosis*, and *M. ulcerans*. There is very little information about the incidence of opportunistic mycobacterial pathogens in most developing countries. Mycobacterium Tuberculosis (Koch bacillus) is the most important mycobacterium species that cause tuberculosis. Tuberculosis also caused by *M. bovis*, *M. africanum* and occasionally by opportunistic mycobacteria.

Some scientist proposed a subdivision of *M. tuberculosis* for epidemiological purpose into five variants,

- 1 *M. TB* var classical human (major type causing human tuberculosis).
- 2 *M. TB* var classical bovine (bovine type *M. bovis* associated with cattle)
- 3 *M. TB* var asian human (human type of Asian origin)
- 4 *M. TB* var afrincan I (west African type of *M. africanum*)
- 5 *M. TB* var afrincan II (east African type of *M. africanum*)

Table 1. Mycobacterial opportunistic and obligate strains with respective pathogenicity.

OPPORTUNISTIC PATHOGENS		OBLIGATE PATHOGENS	
<i>M. Kansasi</i>	Pulmonary infection	<i>M. Tuberculosis</i>	Tuberculosis
<i>M. Szulgai</i>	Pulmonary infection	<i>M. Africanum</i>	Tuberculosis
<i>M. Xenopi</i>	Pulmonary infection	<i>M. Bovis</i>	Tuberculosis
<i>M. Avium</i>	Cervical adenitis	<i>M. Leprae</i>	M.TB Complex MAC Leprosi
<i>M. Scrofulaceum</i>	Cervical adenitis	<i>M. Ulcerans</i>	M.TB Complex MAC Skin ulcer
<i>M. Fortuitum</i>	Skin infection		
<i>M. Marinum</i>	Skin infection Superficial		

M. bovis and *M. africanum* have been given their own individual species names, if the criteria used to classify other organisms were applied, including DNA homology.

Atypical/ anonymous/ environmental

These terms were formerly used to describe those organisms that were not tubercle bacilli. Most of these anonymous organisms have now been given species names and each organism is typical of its own species. It is now recommended, therefore that the terms atypical and anonymous should be discontinued, and the term environmental mycobacteria should be used instead.

Acid fast staining of M-TB

This is the most important characteristic of mycobacteria. It means that when mycobacteria are stained with carbol fuchsin in Ziel-Neelsen technique. They are able to retain (hold fast to) their red color when washed with an acid solution. Other organisms, with the exception of some nocardia species and some corynebacteria, are decolorized by the acid.

Cultural characteristics of mycobacteria

With the exception of *M. Leprae*, mycobacteria can be grown aerobically using a protein-enriched culture media. Many saprophytic mycobacteria and the opportunistic pathogens *M. fortuitum* and *M. chelonae* are able to grow on non-enriched medium i.e. nutrient agar. An atmosphere which contains an increased amount of carbon dioxide has been shown to improve the growth of some mycobacteria. The culture of specimens for the isolation of *M. tuberculosis* is described in subsequent subunits. Some of the cultural features which are used to differentiate mycobacterium species include, a) Rate at which growth occur b) Temperature at which growth occur c) Production of pigment in light and darkness.

Partial resistance to acid or alkali of Mycobacterium tuberculosis

Mycobacteria have a much greater resistance to acid and alkali than other bacteria. Specimens such as sputum and urine that contain contaminations can be treated with acid or alkaline reagent before being cultured for *M. tuberculosis*. Such treatment is intended to kill the commensals with the loss of as few tubercles bacilli as possible. The strength of acid or alkali used must not exceed more than is required to decontaminate the specimen otherwise large numbers of mycobacterial will be killed.

Identification of Mycobacterium

The following investigations together with the cultural features provide a basis for identifying the medically important mycobacteria:

- 1 Growth on medium containing 500 ug/ ml 4(p)-nitro-benzoic acid.
- 2 Nitrate reducing test
- 3 Growth on medium containing 10 ug/ ml thiacezone,
- 4 Tween 81 hydrolysis test
- 5 Arylsulphatase test

Other identification tests for M.TB

Some other typical identification tests performed to particularize the specific *Mycobacterium Tuberculosis* species are as given below,

Table 2. Typical identification tests used to specify the mycobacterial species.

ACID FAST ORGANISM FORM LJ MDIUM	FIRST TEST			IDENTIFICATION
	Pigment	25° C	PNB	
Slow grower 36°C Colonies:2-6weeks	N	-	-	M. tuberculosis Complex
	N	-/+	+	Growth at 44°C Tween 80 hydrolysis test Possibly: M.Xenopi, M.avium intracellular complex, or saprophytes.
	P	+	+	Tween 80 hydrolyses test Growth on thiacetazone Nitrate rduction test Possibly: M. Kensi, M. marinum or M. simiae
	S	+	+	Tween 80 hydrolysis test Nitrate reduction test Arylsulphatase test Passibly: M. scrofulaceum M. szulgai or saprophytes
Slow grower 32°C No growth at 36°C Colonies:2-6weeks	N	-	-	M. ulcerans
	P	+	+	M. marimun Note: on subculture M. marinum will grow at 36°C
Rapid grower at 36°C	N or S rarely P	+	+	Nitrate reductontest Arylsulphatase test Passibly: M.chelonae, M.fortuitum or saprophytes.

In zeil-neelsen stained smears, *M. xenopi* appears as a long pale-staining acid bacillus and *M. avium*-intracellular appears as a small acid fast *coccobacillus*.

Key: N- Nonchromogen, S-Scotochromogen, P-Photochromogen, PNB- 4(p)-Nitro-benzoic acid

Normal habitat of Mycobacterium TB

The main reservoirs for *M.tuberculosis* are infected humans, the organism also occur as a pathogen in animals. It mainly transmitted by infected persons coughing and spitting out bacilli which are then inhaled by others. *M. bovis* is found merely as a pathogen in cattle and occasionally in other animals. Human can become infected by close contact with infected cattle or by ingesting the bacilli in mild form infected animals. Person to person transmission of bovine strains may also occur. The incidence of *M. bovis* in developing countries is thought to be low. *M. africanum* has been found in several countries in East and West Africa.

Chapter 4.

MOLECULAR FEATURES OF MYCOBACTERIUM TUBERCULOSIS

Mycobacterium tuberculosis isolates investigated genotypically for the purposes of as under;

1. Confirm the source or the origin of outbreaks (Mokrousov, et al., 2002),
2. explore transmission in public (Van Rie et al., 1999),
3. confirm cross contamination in the laboratory,
4. identify patient's of exogenous reinfection (S Jasmer, 2002) and
5. study clonal expansion of strains.

Clonal expansion is studied comprehensively in some laboratories. Moreover; an appraisal of the phylogenetic reconstruction and epidemiology has been compiled and published (Bifani, P. J., 2002).

Genotyping/ DNA sequencing

M. tuberculosis isolates can be analyzed by standard spoligotyping methods (Kamerbeek, J., 1997) for rapid assessment. Subsequently, standard IS6110-based RFLP methods can be performed on all isolates. Genetic analysis performed with RFLP and identical spoligotype patterns. The matching of spoligotypes designations assigned according to standard nomenclature (Filliol, 2002). Fingerprint images of RFLP analyzed by using BioNumerics software (BioSystematica) on a Dell workstation and captured by using the expression instrument (Epson). While; the strains analyzed by PCR for the dnaA-dnaN intergenic region of the origin of replication. The primers designed to use as Polymerase chain reaction generated probe, A1 region in dnaA-dnaN (Af region [5_-CGCATCCGTCAGCGCTC CAA] and Ar region [5_-GCCAACTCTTGTCGTAGCCGC]). The solutions containing of 1.5 mM MgCl₂, 50mM KCl, 10mM Tris-Cl (8.3 pH), 0.2mM deoxynucleoside triphosphates (dNTPs) (200M each), primer 1M (each), DNA template 50ng, and 1U of Taq polymerase (of Sigma Chemical Company, St. Louis, Mo.) can be made for last reaction volume of 50 l. The times of Polymerase Chain Reaction cycling maintained

- 4 minutes at 94°C temperature,
- Thirty cycles of 30 second, at temperature of 94°C, 30 seconds at 60°C temperature, and 2 minutes at 72°C temperature, and
- minutes at 72°C temperature.

1% agarose gel used for the purpose of amplification of products analyzed. While; a 537-bp product estimated in samples that not have IS6110 incorporation in the dnaA-dnaN section, as compared with 2,000 bp product expected in those that carried the incorporation. The products amplification confirmed by automated sequencing. Sequencing with an ABI PRISM 377 DNA sequencer (Applied Biosystems, Foster City, Calif.) and QIAquick Spin PCR purification kit (made by Qiagen, Valencia, Calif.) used to purify the products. The sequences were examined through BLAST comparison database of National Center for Biotechnology Information nonredundant sequence. The outbreak strains of principal genetic group were determined by DNA sequencing and PCR amplification of target specific katG and gyrA regions within the genes. While; the primers gyrAf (5_-CACCTACATCGACTATGCGA)-gyrA (5_-GGGCTTCGGTGTACCTCAT) and katGf (5_-TCGACTTGCCGCCCTGACG)-katGr (5_-ACAGCCACCGAGCACGAC) were utilized as described previously. The mixture of PCR have 10mM Tris-Cl

(pH 8.3), 1.5mM MgCl₂, 50mM KCl, 500nM primer (each), 0.2mM dNTPs (200 M each), 1U of Taq polymerase (Sigma), and 50ng of DNA template for a 50 μ l final volume. The times of PCR cycling for katG optimized as follows:

- Four minutes at 94°C temperature,
- 35 cycles of one minutes at 94°C tempratue, 1 minute at 60°C tempratue, and 2 minjutes at 72°C temprature;
- 15 minutes at 72°C temprature.
- While; for the maintainedas of gyrA the procedure is as follows:
- minutes at 94°C tempratue;
- 30 cycles of 1 minutes at 94°C, 1 min at 55°C tempratue, and 1 min at 72°C; and
- 15 minjutes at 72°C tempratue.

The products examined on an agarose gel 1%, and the appropriate product sizes detected. Then; products were sequenced and purified (as explained above) by using the multiplex PCR approach for the determination of IS6110 in the NTF-1 area of the epidemic in the Mycobacterium tuberculosis strains. In short; a reaction mixture 50- μ l containing 10mM Tris-Cl (pH 8.3), 1.5mM MgCl₂, 50mM KCl, 1.0 μ M primer (each), 0.2mM dNTPs (200 μ M each), and 50 ng of DNA template. 1U of Taq polymerase amplified with the following parameters:

- minutes at 94°C temperature;
- 30 cycles of 1.5 min at 94°C temperature, 1.75 minutes at 60°C temperature, and 2.5 minutes at 72°C temperature;
- minutes at 72°C temperature.

Products were examined on an agarose gel 1.5%. there were miscellaneous band sizes expected for different strains (Joy, 2004). We can reviw the medical charts of all tuberculosis diagnosed patients to establish the results of the sputum examination, site of disease and human immunodeficiency virus (HIV) test of each patient's. While; the epidemiological associations among patients can be investigated to determine the results of tuberculin skin tests. The Drug susceptibility testing of *Mycobacterium tuberculosis* isolates performed for all patients. RFLP and Spoligotyping images can be compared independently in Surveillance Network (NTBGSN) database, Centers for Disease Control and Prevention (CDC), National TB Genotyping (Crawford, J., 2002) and the Public Health Research Institute (PHRI) TB Center database in Newark, (S. Joy Milan, 2004).

Genetic basis of resistance

Automated DNA sequencing is the most common-ly applied technique to characterize mutations. The other techniques applied include dideoxy fingerprinting, Polymerase Chain Reaction - Single Strand Conformational Polymorphism (PCR-SSCP), PCR heteroduplex analysis, line probe hybridization and heminested PCR. The newer techniques include RNA-RNA duplex base-pair mismatch analysis, allele specific PCR and DNA sequence analysis using fluorogenic reporter molecules (Molecular beacons). Multidrug-resistant tuberculosis is a worldwide growing health problem (Ramaswamy, and Musser, 1998).

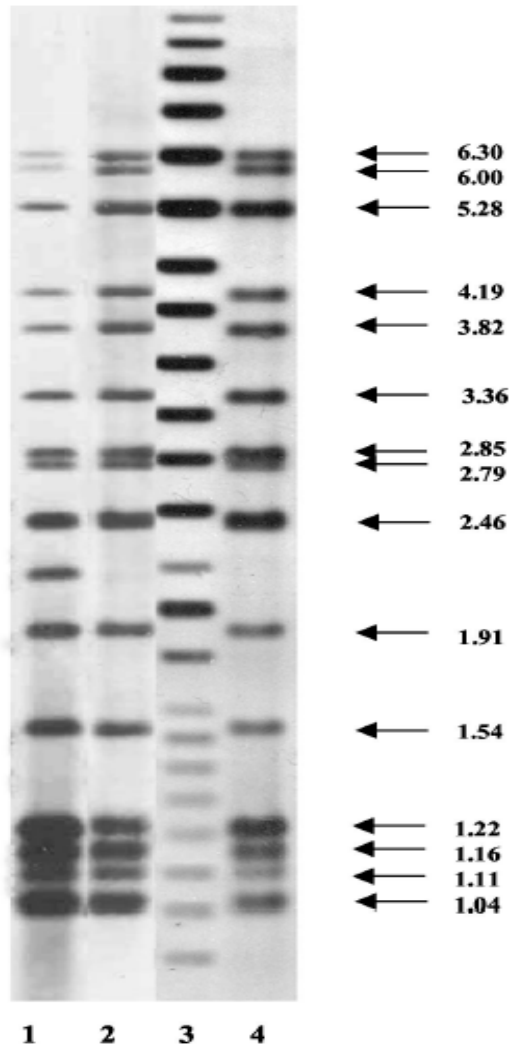


Figure 3. IS6110-based RFLP genotyping patterns of N family strains isolated in New York City and Seattle. Lanes 1 and 2, New York City strains N2 and N4. Lane 4, Seattle outbreak strain SBR19. Lane 3, molecular size standards (in kilobases) (S.Joy Milan et al., 2004)

The significant mortality are associated with MDR-TB (Turett et al , 1995) and resulted in severe outbreaks in institutions (Frieden TR., et el 1996). **Early** treatment and isolation of tuberculosis patients can only be enabled by rapid diagnostic assays especially in multidrug resistant cases. It should also be addresse rapidly to assure successful treatment (Mani, et al , 2003). Resistance againsty rifampin is a good marker for multidrug resistant Mycobacterium tuberculosis. Moreover; 90% of *M. tuberculosis* strains resistant against rifampin are more likely be isoniazid resistant too. Hence, they are classified as multidrug resistant (Torres et al., 2000). Rifampin resistance is also acquiescent to diagnose and detect genotypic assays more rapidly. Because; approximately 95% of rifampin resistant *M. tuberculosis* strains contain *rpoB* mutations localized of 81 bp core region of the bacterial RNA polymerase gene (Riska, et al., 2000). Furthermore; almost all mutations that arise in this region produce resistance against rifampin. On other way; nearly all *M. tuberculosis* isolates susceptible to rifampin have the same wild type nucleotide sequence in this region (Sreevatsan, et al., 1997).

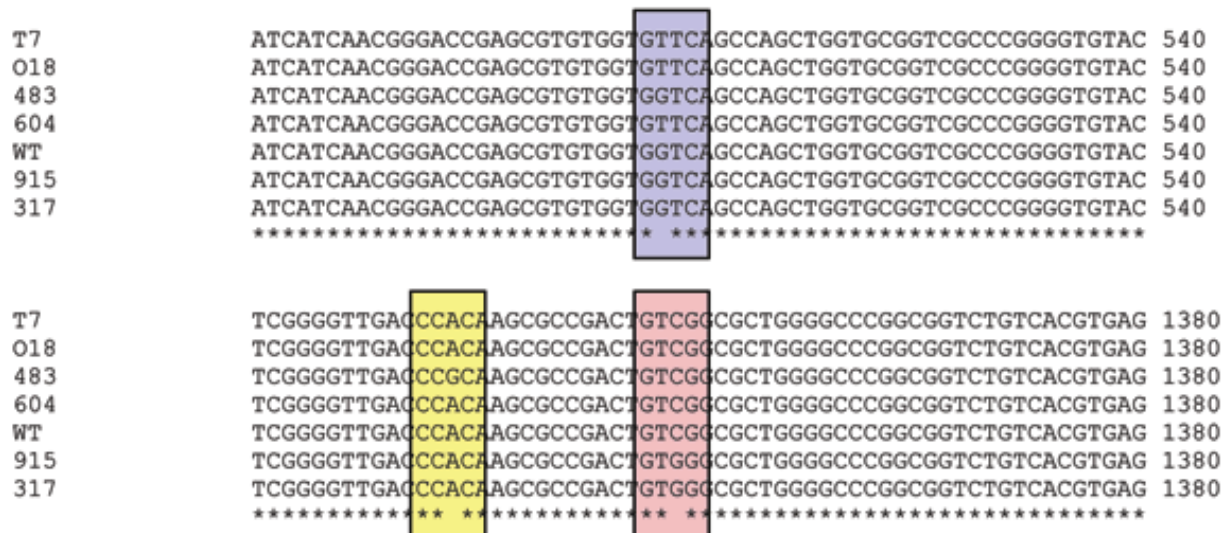


Figure 4. Genotyping of Rifampicin resistant gene of *Mycobacterium TB*.

Restricted fragment polymorphism PCR (RFPCR) and DNA probes

Polymerase Chain Reaction (PCR) and DNA extraction

From Lowenstein Jensen medium a loopful of colony has been taken and emulsified in sterilized water. It was then boiled for 10 minutes, the culture lysate preserved at 20 °C. Approximately 5l was used as DNA and each time the polymerase chain reaction carried out.

Primers

The primers forward and reverse used were rpo 105: 5'CGT GGA ACG GGT GCA GAC GT 3' and rpo 293:5' AGT GCG ACG GGT GCA CGT CGC GGA CCT 3' to carry out the the polymerase chain reaction. The region constitutes a 215 bp amplified product in the rpo B region (Accession No. BX842574). The PCR profile was initial denaturation at 95°C temprature for 5 minutes and 72°C temprature for 1 minute and a final extension of 72°C temprature for 5 minutes.

DNA sequencing

The sequencing of DNA carried out by using ABI 310 Genetic Analyser (Applied Biosystems Inc., Foster City, CA, USA). The forward and reverse Both primers used to confirm the results. The sequence data was then eventually examine and accumulated (P.Deepa et al., 2005). The line probe assay, commercially available INNO-LiPA Rif.TB; Innogenetics NV, Haven, Belgium, executed according to the instructions of manufacturer's, demonstratee a wild type blueprint suggesting invitro sensitivity to different drugs.

Assay conditions

Experimnet related to the PCR can be conducted throuth 96 well microtiter plates manufactured by Applied Biosystems, Foster City, Calif. (El-Hajj et al., 2001). The fluorecence is measured in each well throughout the annealing step during the course of every reaction. To calculate the fluorecence strength contributed by each of differently colored molecular beacons, the spectral data analyzed automatically by the computer program that control the spectrofluorometric thermal

cycler. The mutation within the *rpoB* core region 81-bp can be detected by the absence of a distinctive growing fluorescence signal. While on the other hand; *Mycobacterium tuberculosis* isolates supposed to be susceptible if the molecular beacons assay produce a characteristic rising signal (Mandira et al., 2004).

PCR-SSCP

A variety of molecular methods have been established for the purpose of fast mutations detection in the *Mycobacterium tuberculosis* core region, including the line probe assay (De Beenhouwer et al., 1995), SSCP PCR or Single Strand Conformational Polymorphism (Telenti, A., et al., 1993), and real-time PCR (Torres, et al., 2003). The scientists has developed a molecular beacon based realtime PCR assay for this function (Piatek, 2000) and afterward improved into a multicolor, single tube assay format. The single well molecular beacon assay used 5 molecular beacons. Each hybridizing to a different target segment within the core region and labeled with a differently colored fluorophore. Thus; the designing of every molecular beacon was made to be explicit that it will not bind to its target if that target is sequenced differently. The sequence of wild-type *Mycobacterium tuberculosis* can even be conducted through a single nucleotide substitution (Marras, S, 1999). Hence; the molecular beacons fluoresce only when they are bound to their actual targets. The appropriateness of the single tube molecular beacon assay to identify the *rpoB* gene mutations in the clinical *Mycobacterium tuberculosis* specimen from the high occurrence countries can be assessed (Mandira varma basil, 1999).

Comparison of molecular beacon assay to SSCP-PCR

The resistant and susceptible isolates characterized and investigated by single-strand conformational polymorphism PCR (Bobadilla., et al., 2001). Sequencing of DNA isolate revealed the presence of mutation in the outere region of the core region (Mandira et al , 2004).

Chapter 5.

PATHOGENESIS OF TUBERCULOSIS

Infection is usually a localized multiplication of the organism in the lung and nearby lymph glands. In children there is often a marked enlargement of the lymph glands. This first multiplication of the organism is referred to as the primary tuberculosis infection. Occasionally the primary infection is in the tonsil or intestinal tract. In most people the primary infection is self-healing. The healed area often becomes calcified. Pulmonary tuberculosis can occur when the primary infection does not heal completely and there is either continued multiplication or reactivation of the organism in the lung several months or years later. Reactivation may occur due to poor health, malnutrition or defective immune response. It may also be useful to be aware of the tuberculosis pathogenicity to conceive all the related hazards. Majority of the people usually not get tuberculosis illness, because specific cell mediated immunity usually produced within 2-10 weeks after the initial infection (Karin et al., 1997).

Granulomatous Lesions and Tissues Destructions

The pulmonary tubercular lesion can be divided into five major types,

- Epithelioid granuloma with necrosis.
- Epithelioid granuloma without necrosis.
- Chronic non-specific inflammation.
- Chronic non-specific granuloma.
- Abscess.

Categorization of Patients (on basis of smear results)

- 1st Category: Pulmonary tuberculosis positive patients, Pulmonary/ Extra- Pulmonary – positive & seriously ill patients
- 2nd Category: Pulmonary positive patients having previous treatment failure/ relapse/ defaulting history.
- 3rd Category: Pulmonary / Extra- Pulmonary – positive & not seriously ill patients

Disease Classification (on basis of area of origin)

Tuberculosis can be divided into two major classes – pulmonary and Extrapulmonary tuberculosis as given below,

Pulmonary Tuberculosis In the lung, *M. tuberculosis* causes an inflammatory reaction leading to a liquefied destruction of lung tissue with caseation i.e. breakdown of the diseased tissue into a cheese like mass. The yellow pieces of caseous material contain tubercle bacilli and are often coughed up by the patient in sputum. Patients with advanced infection have difficulty in breathing due to cavities in their lungs. The main symptoms include chronic cough with mucoid/ mucopurulent sputum which may contain blood (haemoptysis). In later stages there is loss of weight, fever, sweating especially during sleeping, tiredness, chest pain and anemia, enlargement of lymph, emphysema. Erythrocyte sedimentation rate (ESR) is raised due to an increase in immunoglobulin (mainly IgG and IgA). Rheumatoid factor is occasionally detected in

the serum. Complications of pulmonary tuberculosis include dry pleurisy or pleural effusion, lung collapse, acute miliary tuberculosis and occasionally the tuberculous meningitis.

Extra-Pulmonary Tuberculosis

The tuberculosis that observed other than lungs referred as extra- pulmonary tuberculosis.

Tuberculosis of Menings

The tubercle bacilli reach the menings in the blood. Tuberculosis meningitis occurs more frequently in infants and young children, as a complication of primary tuberculosis infection. The condition is usually rapidly fatal unless treated at an early stage. It is therefore important for the laboratory to exam carefully the cerebrospinal fluid fro acid fast bacilli, especially when the fluid contains lymphocytes.

GIT & Lymph Tuberculosis

Continued active infection in the lymph glands can lead to the bacilli spreading by way to the lymphatic system and blood circulation to the lungs, pleural cavity, kidneys, bones and joints. An enlarged lymph gland may rupture into one of the bronchi (tubes leading to the lung) and cause acute tuberculosis of the affected lung.

Tuberculosis of renal and urinogenital tract and kidney

Tubercle organisms reach the kidney and genital tract by way o the blood circulation. Infection is often suspected when repeater urine specimens are isolated by routine urine culture. In the late stages of infection there is frequency in passing rune and often hematuria. There is usually a recurring fever. Tuberculosis of the genital tract may cause infertility and pelvic inflammatory disease.

Tuberculosis of Bones and joints

Tuberculosis that is observed by accumulation of mycobacterium into bones and joints. It is not very sufficiently observed but exists with marked symptoms of pain, inflammation activation (TNF).

Miliary tuberculosis

Patients are often acutely ill with fever but a chronic form of the disease can also occur. The disease is usually recognized by seeing widespread fine nodules on the chest X-ray. The infection may be accompanied by a low white cell count or occasionally by a leukemoid reaction especially in elderly patients. The liver, spleen and lymph glands may be enlarged and the meninges may also become infected.

RESISTANCE OF MYCOBACTERIUM TUBERCULOSIS

Mycobacterium Tuberculosis Resistant is a serious threat in its successful eradication and control programmes (Victor, et al , 1997). The natural resistance in Mycobacterium tuberculosis has the ability to undergo spontaneous, slow but constant mutation, resulting in resistant mutant organisms. This natural phenomenon is genetically determined and varies from drug to drug. The probability of spontaneous resistance to the individual antituberculosis drugs is as: Isoniazid : 1 in every 10^6 cell divisions, Rifampicin : 1 in every 10^9 cell divisions, Streptomycin : 1 in every 10^6 cell divisions, Ethambutol : 1 in every 10^5 cell divisions, Pyrazinamide : 1 in every 10^5 cell divisions. Usually, the chromosomal location of resistance to different drugs is not linked; therefore, spontaneously occurring multidrug resistance is extremely rare. Since the probability of naturally mutant's resistant occurrence is very low. While; a large bacterial load in lung cavities needed to be treated for MDR-TB strains to emerge (Karin et al , 1997).

Epidemiology of Resistant Mycobacterium

The disease has assumed alarming dimensions in view of emergence of multidrug resistant tuberculosis (Herendra and Shah, 1998). Drug resistant tuberculosis is a high prevalent phenomenon. In the former Soviet Union, the isoniazid primary resistance rates are as high as 32%; while, the primary multidrug resistance is nearly 15%. The primary resistance to isoniazid in Latin America is different from 20% in the Dominican Republic to 1% in Uruguay, and 7% multidrug primary resistance is high as in the Dominican Republic and in Argentina 5% (WHO, 1998). The reports indicated increasing resistance rates to rifampin and isoniazid, 4-times higher than formerly reported rates for Mexico during 1995 (Sifuentes et al., 1995). As after that, a number of studies have dealt with this issue in miscellaneous settings: semi-urban, rural areas and urban. The widespread finding has been an important rate of primary isoniazid resistance (Kato, et al., 1999) and to the isoniazid and rifampin combination (Garcia et al., 2000). A joint effort of the the Mexican TB control program and Centers for Disease Control and Prevention reported 11% rate of isoniazid primary resistance and 2% of multidrug primary resistance during 2000 (Granich RM, et al., 1997). The high death rates 50% - 80% associated with Multidrug resistant tuberculosis spans a comparatively short time 4 - 16 weeks from diagnosis to demise (Dooley, et al., 1992). In the majority of the countries, MDR tuberculosis has increased and interferes with tuberculosis control programs, especially in developing countries. Where the tuberculosis prevalence rates is high as 48%. The elevated death and infection rates has posed an urgent challenge to detect cases rapidly and make sure the successful eradication (Iseman, et al., 1992). The primary resistance is also usually less severe than acquired resistance because of the level of resistance is lower and fewer drugs are usually concerned (Karin, et al., 1997). Multi Drug Resistance tuberculosis strains can arise as an outcome of sequential accumulation of mutations. That conferred the resistance by a single step process (i.e. acquisition of an MDR element) or by single agents. An example of multi drug resistant Mycobacterium tuberculosis strains in New York City, provided by the examination of the evolution of two closely related designated subclones strain W1 and W. There are greater than 300 tuberculosis cases are derived by these two related organisms in New York City and elsewhere. Sequencing of automated DNA has represented organisms defined the correct sequence of distinctive mutations conferring resistance against Rifampin, isoniazide, Ethambutol, Streptomycin, ethambutol, pyrazinamide, quinolones and Kanamycin. These strains resistant against anti-TB drugs have spread to other cities of Europe and US. A number of these MDR samples arise because genetic random mutations that targets encode for the individual antituberculosis agents are selected by sub-therapeutic

medicines levels resultant from poor adherence to treatment protocols, treatment errors or other factors. Multidrug resistant *Mycobacterium tuberculosis* is an emerging dilemma for public health of great importance. That occurred with higher mortality rates than drug sensitive tuberculosis, specifically in patients with poor immunity. Multi Drug Resistance tuberculosis patients require treatment with second line drugs; that incurring higher costs due to prolonged hospitalization, more toxic and remain infectious for longer than patients with drug sensitive strains (Cohn, et al., 1997).

Factors Contributing to the Developing the Resistance

There are clinical, biological, social and epidemiological risk factors that contribute to develop drug resistance. Suboptimal doses, omission of one or more prescribed agents and poor absorption are the main reasons for emergence of drug resistance. Antituberculosis medicines are freely obtainable in the market. That leads to self treatment and improper regimens. While; the appearance of acquired drug resistance is primarily because of the inaccurate regimes application. Monotherapy, poor drug absorption, sub-optimal doses of the drugs and insufficient active agents in the regimen leads to reversion of one or multiple drugs resistance from susceptible within a span of a few months. Acquired drug resistance may be caused due to selection of pre-existing mutants and their eventual proliferation because of inaccurate chemotherapy (Herendra Thakker & Sah JR, 1998).

Clinical factors

Unreliable treatment regimens - Inadequate dosage/duration of drugs. - Free availability of anti-TB drugs over the counter, poor drug supply at treatment centers, and delay in diagnosis.

Biological factors

Biological factors are large bacillary population, Genetic predisposition, local factors in host, cavitory lesion, and insufficient drug concentrations in tissues.

Social factors

Failure of public health system, neglect of disease, irregular or inadequate intake of drugs, ignorance, and poverty are the major social factors that contribute in development of resistance.

Epidemiological risk factors for drug resistance

History of previous anti-tuberculosis therapy, length of previous chemotherapy, contact with a known case of Multi Drug Resistance tuberculosis, living in an area with high occurrence of drug resistant tuberculosis - HIV infection, and social instability (Herendra Thakker and Shah JR, 1998)

Chapter 7.

MOLECULAR BASIS OF RESISTANCE

The likelihood of mutation or resistance is directly proportional to the bacterial load. The 10^9 bacillary load will contain numerous mutants resistant to anyone of tubercular drug (Bodmer T, et al., 1995). Since the mutations impart resistance against drugs are chromosomal, therefore; the probability of mutation is simultaneously occurred in more than one drugs. Thus; the likelihood of MDR is multiplicative (Miriam Bobadilla, et al., 2001). The molecular and genetic mechanisms may involve in the acquisition of Mycobacterium Tuberculosis resistance against drugs. That is concomitant with the development of different molecular strategies of rapid detection of multi drug resistance tuberculosis (Ashok et al., 1998).

Rifampin

This antitubercular drug was introduced in 1972. In resistance; it is extremely effective against Mycobacterium tuberculosis. Rifampicin has MICs of $0.1\mu\text{g}$ - $0.2\mu\text{g}$ (Mitchison and Nuna00, 1985). Rifampicin with isoniazide forms the backbone of short term chemotherapy because of its high bactericidal action (Kochi, et al., 1993). It was a highly effective bactericidal first line tuberculosis medicine and key component of the initial anti-tuberculous regimen. The clinical isolates of Mycobacterium tuberculosis usually having the rpoB rifampin resistant gene in the 81-bp core region that encodes the B subunit of RNA polymerase (Snider & Roper, 1992). In susceptible organisms these mutations are not noticed. Even though; insignificant discrepancies are reported in general, that has a strong correlation with MIC and specific amino acid substitutions. Codons 513, 526, or 531 missense mutations that occurred in elevated Rifampin resistance level. While; the change in amino acid position at 514 or 533 usually produce in low Rifampin resistance levels. In 4% of Rifampin resistant tuberculosis isolates, the molecular mechanism of resistance observed is the lack of RRDR unknown changes (Pearson et al., 1992). This is investigated that approximately 90% rifampin resistant clinical isolates in various areas are resistant to isoniazid, making resistance against rifampin a helpful substitute marker for multidrug resistance. That also representative of an urgent requirement of 2nd and 3rd line drugs for susceptible Mycobacterium tuberculosis (Edlin, et al., 1992). RNA polymerase, a complex oligomer composed of β , β' and, encoded by rpoA, rpoB, rpoC, and rpoD; four different subunits, is highly conserved among bacterial species (Ovchinnikov, et al., 1981).

Table 3. Mechanisms of Drug Resistance in Mycobacterium Tuberculosis

Antimycobacterial agent	Mechanism of action	Resistant Gene	product	Mechanism of resistance
Isoniazid	Inhibition of mycolic acid biosynthesis	(1) KatG (ii) inh A synthesis, (iii) ahpC	Catalase Peroxidase Enoyl-ACP reductase Alkyl hydroperoxide reductase	(i) Mutations in KatG result in failure to generate an active intermediate of EMH. (ii) Overexpression of inhA allows continuation of mycolic acid (iii) ahpC mutations may serve as a marker for lesions in KatG Mutations in rpoB prevent

Rifampicin	Inhibition of transcription	rpoB	B subunit of RNA Polymerase	interaction with Rifampicin
Streptomycin	Inhibition of protein synthesis	(I) rpsL (ii) rrs	Ribosomal protein S12 16s rRNA	Mutations prevent interaction with Streptomycin. Overexpression or mutation of
Etharabutol	Inhibition of arabinogalactan biosynthesis	EmbCAB	Arabinosyl transferase	embAB allows continuation of arabinan biosynthesis.
Pyrazinamide	Unknown	PcnA	Amidase	Loss of pyrazinamidase activity results in decreased conversion of Pyrazinamide to pyrazinoic acid, the putative active moiety (?) Mutations in gyreA prevent interaction with fluoroquinolones.
Fluoroquinolones	Inhibition of the DNA gyrase	Gyra	DNA gvrse sub-unit A	

In *E. coli* the characterization of the *rpoB* gene has demonstrated that rifampicin particularly interacted with the β subunit of RNA polymerase. Thus; hampering the mutations and transcription in the locus of *rpoB* gave conformational changes that lead to the defective binding of the medicine and consequently resistance (Jin and Gross, 1988). Consequently, the Mycobacterial tuberculosis *rpoB* locus was characterized and mutations occurred to contribute the resistant trait (Williams, et al., 1994). Moreover; it is speculated that supplemental mechanisms i.e. mutations in alternate subunits of RNA polymerase and rifampicin permeability may be implicated in conferring the phenotyping of resistance (Kapur et al., 1994). The mutations consistency of *rpoB* locus and the rifampicine resistant phenotype has manifested clinical implications (Rouse, et al., 1996). Since; it may act as a substitute indicator for Multidrug Resistance tuberculosis. Rifampicin resistance has provoked development of a variety of diagnostic trials to improve the sensitivity of mutation recognition (Heym, et al., 1995). Even though; automated sequencing has been applied unambiguously to characterize rifampicin resistance associated mutations. Therefore; a number of other techniques i.e dideoxy fingerprinting (Felmlee, et al., 1995), PCR-SSCP (Pretorius, et al., 1995), (Jaber, et al., 1996), heminested PCR (Whelen, et al., 1995), PCR heteroduplex analysis (Williams, et al., 1994), and line probe hybridization (Cooksey, et al., 1997) are successfully used to identify these mutations. Such novel strategies have been described elsewhere to detect drug-resistant Mycobacterium tuberculosis (Telenti and Persing, 1996). The value of PCR-SSCP analysis has been increased in detection of mutations responsible of drug-resistance. Particularly; the development of nonisotopic PCR-SSCP analysis has enhancing its utility in routine laboratories and simplified the procedure (Kalia, et al., 1997).

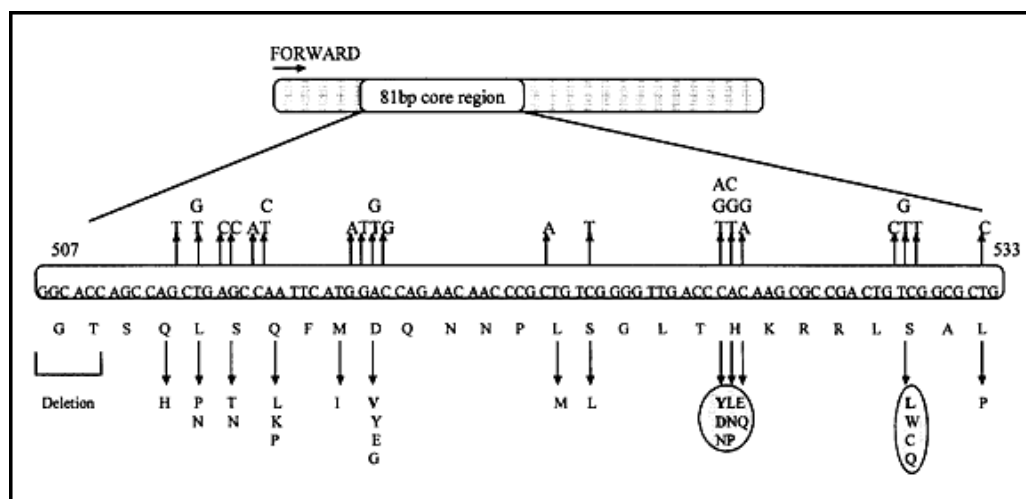


Figure 5. Single amino acid substitutions in the 81 bp core-region of the *rpoB* gene responsible for conferring rifampicin (RIF) resistance (Insertions and deletions that confer the RIF-resistance phenotype are not depicted). Amino acids are represented with single letter abbreviations. Changes in codon Ser531 and His526 account for more than 70% of the mutations with RIF resistance (depicted in shaded ellipses).

The results obtained with SSCP analysis however can be interpreted carefully as the methods only identify mutations and provides no information of type of associated mutation i.e in the *rpoB* gene the silent mutations have been recognized that provide modified mobility models on SSCP examination but have no relationship with rifampicin resistance. That underlines the requirement of caution in interpreting phenotypic, outcomes or genotypic correlation (Kim, et al., 1997).

Table 4: Mutations of the *rpoB* gene found in 35 rifampicin-resistant *Mycobacterium Tuberculosis* isolates

Mutated <i>rpoB</i> codon	Specific Mutation	Strain n	MIC (µg/mL)	p ^c
513	CAA/AAA(Gln/Lys)	1	2,048	0.01
	CAA/AAA(Gln/Lys)	1	256	0.01
	CAA/CCA(Gln/Pro)	1	2,048	0.01
516	GAC/GTC(Asp/Val)	5	37,118	0.01, 0.002
526	CAC/TAC(His/Tyr)	1	256	0.01, 0.002
	CAC/TAC(His/Tyr)	1	1,024	0.01, 0.002
	CAC/TAC(His/Tyr)	3	2,048	0.01, 0.002

	CAC/GAC(His/Asp)	2	256	0.01, 0.002
	CAC/GAC(His/Asp)	1	128	0.01, 0.002
	CAC/GAC(His/Asp)	1	2,048	0.01, 0.002
531	TCG/TTG(Ser/Leu)	1	32	0.002
	TCG/TTG(Ser/Leu)	1	64	0.002
	TCG/TTG(Ser/Leu)	4	256	0.002
	TCG/TTG(Ser/Leu)	1	1,024	0.002
	TCG/TTG(Ser/Leu)	3	2,048	0.002
	TCG/CCG(Ser/Pro) ^b	1	8	0.002
	TCG/GCG(Ser/Ala) ^b	1	64	0.002
	TCG/GCG(Ser/Ala) ^b	1	128	0.002
	TCG/TGG(Ser/Trp)	1	512	0.002
	TCG/TTC (Ser/Phe) ^b	1	2,048	0.002
522	TCG/TTG (Ser/Leu)	1	8	
533	CTG/CCG (Leu/Pro)	1	2	
572	ATC/TTC (Ile/Phe) ^a	1	2	
^a This mutation is located outside the core region. ^b Not previously described. ^c Mann-Whitney test.				

Isoniazid

The isonicotinic acid hydrazide, 4-pyridinecarboxylic acid hydrazide or isoniazid is a synthetic, bactericidal agent and highly active against *Mycobacterium tuberculosis*, *M. microti*, *M. africanum* and *M. bovis* used as a 1st Antituberculosis drug therapy, prophylaxis and laboratory investigation. It has extremely low MIC of 0.02 µg/ml to 0.06 µg/ml (Cangelosi, et al., 1996). The mechanisms of conferring isoniazid resistance are not completely understood and very complex. That has not yet been understood about the mode of action and bacteria targets of isoniazide. Scientists reported small deletions, mutations or insertions that are not characterized in strains of isoniazide sensitive control. Mutations leading to isoniazide resistance have been identified in different gene

targets including *inhA*, *KatG*, *ahpC* and other genes that remain to be established. *Mycobacterium Tuberculosis* isolates by PCR and found the mutation frequencies for isoniazide resistant strains are *inhA*, *KatG*, *ahpC*, *KatG-inhA* and *KatG ahpC*. Amino acid replacements in the NADH binding place of *InhA* apparently result in isoniazide resistance by preventing the mycolic acid biosynthesis inhibition. *KatG* mutations or *inhA* mutations do not considered for all isoniazide resistant strains because 15-25% isoniazide resistance clinical specimens have both *inhA* and *KatG* wild type genes. The isoniazide resistance mechanism in some mycobacterium strains remains to be determined (Julio, 1997). The evidence propose that isoniazide inhibits the synthesis of mycolic acids - the long-chain, branched β -hydroxylated fatty acids of cell wall. Thereby; makes the mycobacterium susceptible to reactive oxygen radicals or other environmental factors. The activation of isoniazide to an electrophilic unstable intermediate needs the enzyme *KatG*, coded by *katG* (catalase peroxidase) and hydrogen peroxide (an electron sink) (Hirano K, et al., 1999). Although; the spontaneous decomposition of isoniazide to form hydrazine may contribute to mediate/ activate isoniazide (Maggliozzo RS, et al., 1996). However, only the *KatG* is the enzyme capable of activating isoniazide. The *KatG* mutant *Mycobacterium tuberculosis* strains are consistently resistant to isoniazide. The Middlebrook's early studies demonstrated that isoniazide resistance may be associated with loss of catalase activity (Banaiee, et al., 2001). While; the genetic studies confirm that transformation of isoniazide resistant of *M. smegmatis* and *M. tuberculosis* strains with restored functional *KatG* isoniazide susceptibility put forth the assumption that *katG* deletion may cause resistance against isoniazide in *M. tuberculosis* (Zhang, et al., 1993). Nevertheless, in absence of inducible genetic response of peroxide is executed in the most of the bacteria by the transcription factor *OxyR*, *KatG*. That is the only peroxide inducible *Mycobacterium tuberculosis* protein (Sherman, et al., 1995). Therefore, *Mycobacterium tuberculosis* resistance to isoniazide is paradoxical; it has to sacrifice *KatG* function. *Mycobacterium tuberculosis* ability to adjust with the loss of *KatG* function or fight organic peroxides is noteworthy. Thus; description of the *oxyR- ahpC* region additionally verified that mutations accountable for *AhpC* upregulation taken place at low frequencies and mainly G>C to A>T transitions limited to a small area in the *oxyR-ahpC* dominant section (Sreevatsan S, et al., 1997). While; the alterations in sequence *oxyR- ahpC* region predominantly limited to resistant against isoniazide clinical isolates. Moreover; there are not all modifications noticeably improved the *AhpC* levels. Mutations can be disrupted by the *katG* gene; leading to the invention of gene product with compromised peroxidative activity or an inactive gene product. Amplification of *katG* gene by PCR followed by single strand conformational polymorphism identify mobility shifts supporting the existence of these mutations and thereby resistance against isoniazide. The discovery of the *inhA* locus eventually determined to be the factors involved in resistance to isoniazide that led as the main target for co-resistance to ethionamide and isoniazide (Wilson, et al., 1996). Two open reading frames (ORFs) compose this locast and designated *inhA* and ORF1, separated by a 21-bp noncoding region.

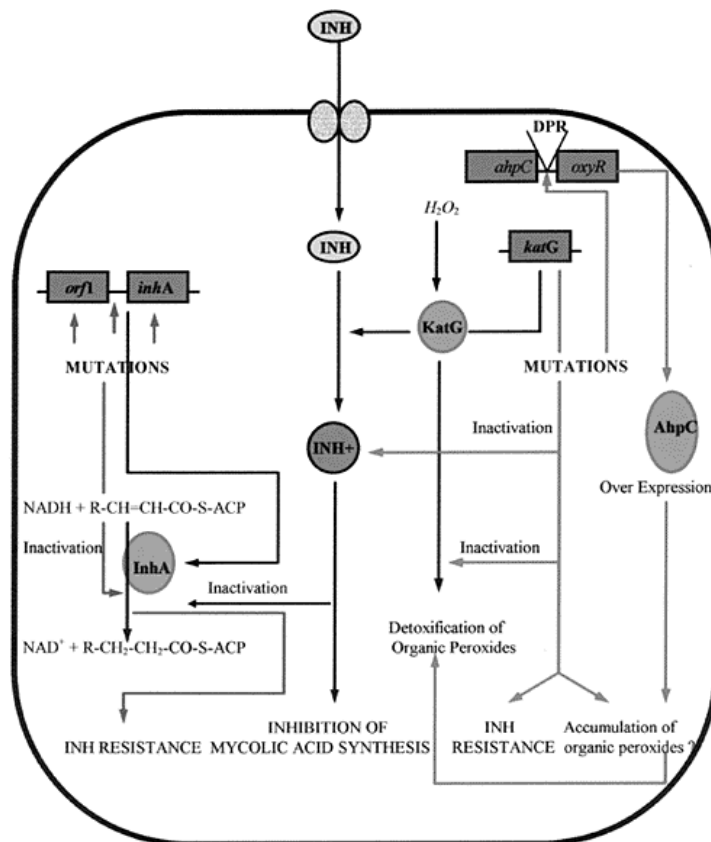


Figure 6. Mechanism of action of isoniazid (INH); acquisition of resistance and combating oxidative stress. DPR, divergent promoter region.

An enoyl-ACP reductase Inh A is probably 40% similar to the EnvM protein, that catalyzes an early step in fatty acid synthesis among enterobacteria (Bergler, et al., 1994). Like InhA, EnvM; activity is thought to employ NAD(H) as cofactor. Consequent biochemical description of InhA function confirmed that it catalyzed the reduction of 2-trans-octenoyl-acyl carrier protein. That is the protein of enoyl CoA esters (Johnsson K, et al., 1995), acting in chain elongation at the final step of synthesizing the fatty acid (Quemard A, et al., 1995). This study disagrees with previous biochemical substantiation suggesting the involvement of an enzyme in unsaturated 24-carbon fatty acid synthesis target of activated isoniazide. Therefore, the biochemically identified targets and complementation of *M. smegmatis* are dissimilar. Lipid pulse labeling trials confirmed the lipid biosynthetic response of *Mycobacterium tuberculosis* and *M. smegmatis* after when exposed with isoniazide (Mdluli, et al., 1996). That designated an unlike mechanism of action for the isoniazide intermediate in the two species. While; the purposeful characterization of inhA mutations, that taking place with katG mutations -as seen in specimens possess very high MICs, in relation to lipid metabolism of isoniazide resistant isolates. That may probably resolve this inconsistency and describe the functions of the relevant loci in the isoniazide's mechanism of action. It subsequently acquires the drug resistance.

Pyrazinamide

Pyrazinamide is analogue (structurally) of nicotinamide; used as a 1st tuberculosis drug. In 1952, it was considered as antituberculosis, however; in the mid- 1980s, it became significant part of short term therapy.

Pyrazinamide is not affected by any other drug and active against semidormant bacilli. It possesses strong synergy with rifampicine and isoniazide and reduces the chemotherapeutic program for antitubercular therapy six months from nine to twelve months. Depending on the conditions applied, MICs of pyrazinamide is being different from 8µg/ml - 60µg/ml. It has no important bactericidal results and is mainly supposed a "sterilizing drug" (Heifets and Lindholm, 1992). The acidic environment is supposed to be phagolysosomes the tubercle bacilli make pyrazinamidase. That enzyme produces pyrazinoic acid from pyrazinamide, the active derivative of this compound. The *Mycobacterium tuberculosis* *pncA* gene encoding pyrazinamidase is sequenced to describe the molecular based mechanism of pyrazinamide resistance. The outcomes provided proofs of *pncA* mutations gave pyrazinamide resistance. The DNA of pyrazinamide resistance clinical isolates sequenced to identify mutations at codons, 162, 141, 138 and 63. While on the other hand; susceptible *Mycobacterium tuberculosis* has sequences of wild type. In 28% of pyrazinamide resistant isolates lack of *pncA* mutations recommended the presence of minimally one extra gene contributing the resistance. An amazingly broad range of *pncA* mutations resultant in structural modifications in the PncA has recognized in higher than 70% of drug resistant specimens. It is supposed that these structural modifications detrimentally change role of enzyme. In this manner; pyrazinamide altered to its bioactive form (Mitchison DA. 19985).

Ethambutol

Ethambutol or synthetic compound dextro-2,2'-(ethyldiimino)-di-1-ol has profound antimycobacterial activity. It is a 1st Antituberculosis drug with a broad spectrum of activity, different isoniazide. Ethambutol is also promoter in dispersed infections of *M. avium* complex, especially in HIV infected patients (Masur H., 1993). Mechanism of action and genetic basis of resistance of ethambutol were largely difficult to understand. Specificity of ethambutol for *Mycobacterial* species has though, pointed out that its target may be implicated in the creation of the external cell wall structure. Synergy consequential from coadministration of ethambutol and other drugs gave additional confirmation for the contribution of ethambutol to hinder the development of cell wall. It is a 1st antituberculosis bactericidal drug. That has been anticipated to be an analog of arabinose. Its specifically target is arabinosyl transferase, most probably a functionally significant place. A two gene locus (*embAB*), encodes arabinosyl transfer has been established to understand the mechanism of resistance of ethambutol. These regions of clinical isolates Automated sequenced from diverse geographical areas, which discovered 69% ethambutol resistance of clinical isolates having an amino acid substitution in *EmbB*. That was not seen in strains susceptible to ethambutol. However; the mutations were also recognized in three further codon 285, 330 and 630. While; the majority of mutations in codon 306 were noticed in *MTB* strains. These mutations were also exclusively characterized among organisms resistance to ethambutol. The informations are steady with the proposal that particular amino acid substitutions in *EmbB* detrimentally influence the interaction between *EmbB* likely to be an arabinosyl transferase and ethambutol, a putative arabinose analogue. Ethambutol resistances are associated with *EmbB* mutations in approximately 70% of ethambutol of *Mycobacterium tuberculosis*. The basis of ethambutol resistance in many organisms missing mutations in ERDR of *EmbB* is unidentified (Julio, 1997). Attachment of mycolic acids to the 5'-hydroxyl groups of D-arabinose residues of arabinogalactan form mycolyl-arabinogalactan-peptidoglycan complex in cell wall. Interruption of the arabinogalactan synthesis hinders the development of this complex. That may lead to improved permeability of the cell wall. Consequently, it was confirmed that ethambutol particularly inhibited arabinogalactan production (Takayama, et al., 1989). An advancement was attained in describing the precise cellular target for ethambutol with the identification and isolation of DPA (β-D-arabinofuronosyl - 1 - monophosphoryl decaprenol), that accumulates fastly, less than 2 minutes on exposure of ethambutol sensitive cells to ethambutol. DPA is a cell free assay systems of arabinosyl donor and one of the main

intermediates of arabinan synthesis method. It was afterward exposed that Ethambutol in particular inhibited arabinosyl transport, proposing arabinosyl transferase as a main cellular target for ethambutol. Detection of arabinosyl transferase as the main target for ethambutol supports to disentangle the ethambutol resistance at genetic level. From an ethambutol resistant strain of *M. avium* emb locus was cloned by utilizing the target overexpression of plasmid vector (Belanger, et al., 1996). While; description of the embR ORF demonstrated that the region was amazingly homologous with a Streptomyces family of transcriptional activators and therefore can modulate the embA and embB expressions. The mapping investigations have additionally confirmed that both embA and embB, were essential to ethambutol resistance alongwith the different promoter region. The proteins of embCAB are supposed to be essential membrane proteins, with consistent role in the synthesis of different arabinan linkage motifs of the lipoarabinomannan and arabinogalactan (Telenti, et al., 1997).

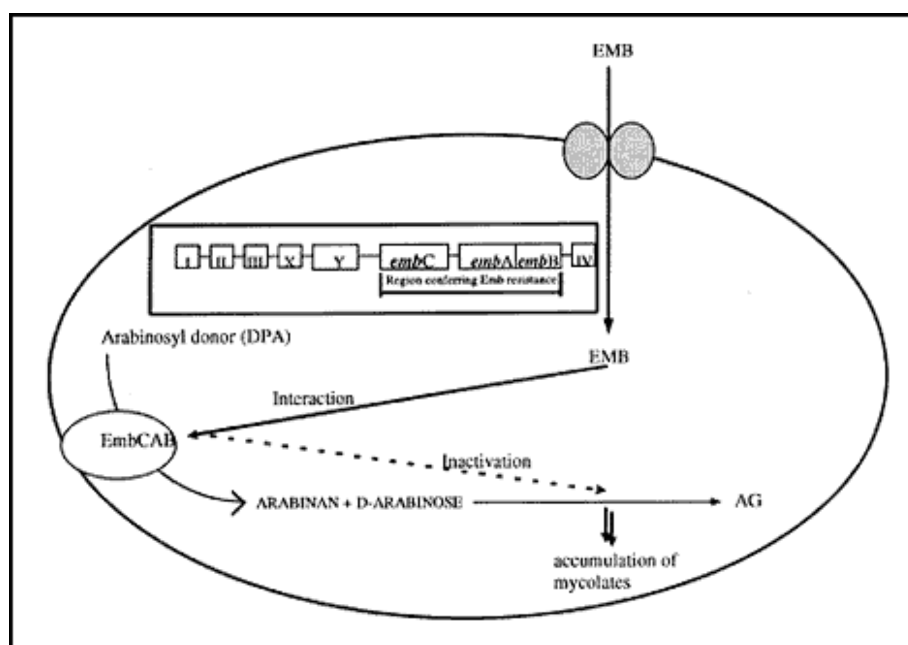


Figure 7. Mechanism of action of ethambutol. EMB interacts with the EmbCAB proteins encoded by the *embC*, *embA*, and *embB* genes, leading to inactivation of arabinogalactan synthesis. Mutations in the *embB* locus cause alterations in EmbB, possibly leading to an altered target for EMB.

Alternatively, hyperexpression of the EmbCAB proteins could lead to EMB resistance. Inlet box:

**Organization of the *emb* operon in
Mycobacterium Tuberculosis (MTB).**

Detection of the embCAB genes provoked a comprehensive investigation of the molecular mechanisms of ethambutol resistance development in Mycobacterium tuberculosis isolates.

Prevention of the Development of Resistance.

Some valuable steps are taken to cure and reduce the resistance. That can assume alarming proportions. Planned and supervised therapy with judicious surgical interference is the only way to ensure cure for tuberculosis patient or prevent the spread of drug resistance (Herendra and Shah, 1998).

Test Procedures Used for Drug Sensitivity/ Resistance for MTB

There certain methodologies used to determine the susceptibility of mycobacterium tuberculosis. Which are given below,

Radiometric method

Radiometric BACTEC (Becton Dickinson) method of detection of mycobacterial growth by sophisticated gamma camera

Agar proportion method

Miscellaneous proportions of drug incorporated in media to calculate the MIC of particular antitubercular drug. 2.7.2.3 MGIT method: Microbial growth indicator tube method used to identify the susceptible/ resistant Mycobacterium tuberculosis. 2.7.2.4 LJ Proportion method: Lowenstein Jensen medium that is specific for mycobacterial growth is used to evaluate the susceptibility/ resistance of mycobacterium. It may also be used to determine the level of resistancy of resistant mycobacterium tuberculosis.

Disk elusion method

The disks with different drug concentrations used to determine the accurate MIC of specific antitubercular drug. It is usually not recommended to mycobacterial species because of some technical discrepancies.

Microtitre method

A minute quantity of mycobacterial/ antigenic substance used to produce a reaction with a given volume of substance to characterize/ determine the specific parameter.



Figure 8. Growth pattern (colonies) of *mycobacterium* Tuberculosis on LJ medium

BACTEC 450

In Becton Dickinson system a liquid medium prepared to grow the Mycobacterium Tuberculosis rapidly that contains the radio-labelled palmitate as the sole carbon source. The growing/

multiplying mycobacterium use and break down the palmitate and liberates radiolabeled CO₂. Using the BACTEC system MTB can be detected in 9-16 days rather than more the four weeks while using conventional media.

Luciferase Assay

Luciferase is rapid assay for drug resistant bacteria. It enzyme is obtains from fireflies that produce flashes of light in ATP presence. Less amount of ATP will be produced and less light be flashd if organism is sensitive to medicine.

Chapter 8.

DIAGNOSIS AND PROGNOSIS

Application of accurate chemotherapy and early diagnosis are important because as under;

- 1 Development of resistance;
- 2 Cause unnecessary drug toxicity;
- 3 Decrease chances of cure; and
- 4 Increase the cost of therapy (Herenra and Shah, 1998).

More than a century back; the discovery of the causative agent and the bacteriological diagnosis of tuberculosis were main obstacles in the control and treatment of tuberculosis. Because of its worldwide occurrence, the methods for diagnosis were under rapid development (Jain, 1996).

Patients personal and medical history

A prompt diagnosis and implementation of effective chemotherapy is the mainstay in the drug resistant management of tuberculosis. Any history of contact with known drug resistant patient should arouse suspicion. The drugs history (previously taken medication) should be recorded vigilantly, because most of the time an accurate history play a key role in diagnosis. Drug history recorded under the heading of (a) drugs used irregularly and inadequately or as monotherapy (b) drugs used regularly in adequate doses for short periods (c) drugs never used (d) cross resistant drugs. If drugs have been used irregularly in inadequate doses or as monotherapy, the chances of patients becoming drug resistant are high. If drugs were used regularly in adequate combination and doses, the chances are that the patient is still sensitive to drugs.

Patient's Physical (Symptomatic)

Examination Physical symptoms include anorexia, weight loss, fever, sweating, weakness, sputum with/ without blood, respiratory complications, chest pain etc.

Clinical evaluation

Presence of drug resistance should be suspected when (i) No clinical improvement is evident in the patient in spite of adequate chemotherapy and regularity during three months; (ii) Presence of giant/ multiple cavities and lung destruction; (iii) Sequential x-rays showing deterioration or no improvement after three months of therapy (iv) Fall and rise phenomenon may occur in which direct sputum smear examination initially shows a fall in number of bacteria due to killing of susceptible strains. This is followed by a gradual rise in count due to growth of resistant organisms that remain unaffected by the drug. The presence of this phenomenon indicates drug resistance, (v) Suspect presence of drug resistance when patient is on regular and adequate chemotherapy but sputum for Acid Fast Staining Bacilli (AFB) by direct smear is affirmative even after five months of tuberculosis therapy, (vi) Immunocompromised person or person with HIV positive infection may develop drug resistance. The clinical parameters are more important and informative for diagnosis instead of exclusive dependence on sophisticated and costly laboratory investigations. Traditional culture and sensitivity test: The drug resistance is confirmed by obtaining

the culture and sensitivity test. The sensitivity culture test of the mycobacterium tuberculosis can be determined with the assistance of either LJ Media or 7H10, 7H11 Middlebrook. Therefore; if person is smear positive. Then; it is gone through the determination of pattern of resistance by incorporating the drug into the LJ medium. Eventually; the results are obtained within 6-8 weeks. While; in non tuberculosis or sputum negative patients, culture of the *Mycobacterium* is necessary and susceptibility tests of cultured bacilli are carried out. This generally requires 12-16 weeks. While; the traditional techniques delay the treatment and time taking; thus at least need 2-4 months. The rapid and newer methods of sensitivity and culture have been established.

Slide culture

Mycobacteria are fixed on slide and then transferred to the liquid medium containing the drug. This is incubated at 37° degree C for one week and the growth is examined microscopically. It gives sensitivity results within 8 to 10 days.

Egg enriched sheep blood media (EESBM)

In this, egg enriched sheep blood medium is used instead of LJ medium for rapid isolation of organisms by using slide culture, when sputum positive samples are used, the outcomes of drug susceptibility are obtainable within 8-10 days. The results of culture and sensitivity tests of mycobacteria by using EESBM are almost at par with those of traditional culture as LJ medium.

BACTEC System

It is radiometric detection of mycobacterial growth with the help of sophisticated gamma camera by using radioactive ¹⁴C. It gives results of culture and sensitivity within 10-14 days

Luciferase Reporter Mycobacteriophage Test (LRM Test)

This is one of the newer and rapid methods used for accurate culture and susceptibility testing. This test uses mycobacteriophage, a virus that infects *M. tuberculosis*, and has a cloned gene for production of luciferase reporter enzyme. The LRM (Luciferase mycobacteriophage) when mixed with culture of bacterial cells results in production of light. The procedure of the test includes growth of mycobacteria in the drug treated medium to which LRM particles are added and then emitted light is measured. If drug kills the mycobacteria no light is produced, revealing sensitivity of the organism to the drug. If mycobacterium is resistant to the drug, the cell remains unaffected and light will be produced. This test is extraordinarily sensitive, specific and rapid. It provides results of sensitivity of *M. Tuberculosis* to various drugs within 48 to 72 hours.

Restriction fragment length polymorphism (RFLP)

RFLP is used to classify and compare the clinical isolates of *Mycobacterium tuberculosis*. Patients isolates can be compared and can be categorized into drug sensitive/multiple drug resistant and treatment can be started on that basis.

DNA Finger printing

It has been confirmed that during development of resistance against drugs, DNA is usually be changed.

Single stranded Confrontation Polymorphism in conjunction with PCR (SSCP PCR)

This test is very useful for primary drug sensitivity. isoniazide, RMP, SM resistance can be performed directly and gives results in 48 hours. Currently this test is being tried.

Ligase chain reaction

The enzyme DNA Ligase is utilized in this test to join two strands of DNA as double strand. Thus; ligase chain reaction method it is possible to detect mismatch of nucleotide. (Jain, 1996)

Radiological Examination (X- Rays Abnormalities)

X ray evaluation used to determine the outcome the treatment protocol/ regimen. If certain abnormalities retained, the therapeutic profile reviewed and may be changed.

Acid Fast Staining/ Microscopic Examination

The pulmonary tuberculosis can be diagnosed be made by the identification of acid fast bacilli by microscopy method, using fluorochrome stain and/or carbol fuchsin stain. Microscopic method is a rapid but be deficient in adequate sensitivity. Therefore; it does not differentiate among various mycobacteria species. The microscopy sensitivity is not often $\geq 25\text{-}40\%$ as compared with culture. However; under ideal environment, it may be possible to attain 60-70% rate. Microscopy method is still the diagnostic and preventive base for tuberculosis control programme in developing countries. The carbol fuchsin stain method is not supposed be better than fluorochrome stain technique; however it is less specific, expensive and not suitable to use as a everyday procedure (Jain, 1996). The sputum from the tuberculosis suspected patient processed to acid fast staining. These acid fast bacilli examined under microscope. The growth pattern and number of colonies are counted.

Fluorescent staining

In this type fluorescent staining of staining, Auramine Stain is used which can be visualized by fluorescence microscopy.

False acid staining

There is no actual tuberculosis but the specimen indicates a positive test results. False negative AFB staining: The negative AFB test result of a tuberculosis positive patient called false acid fast staining.

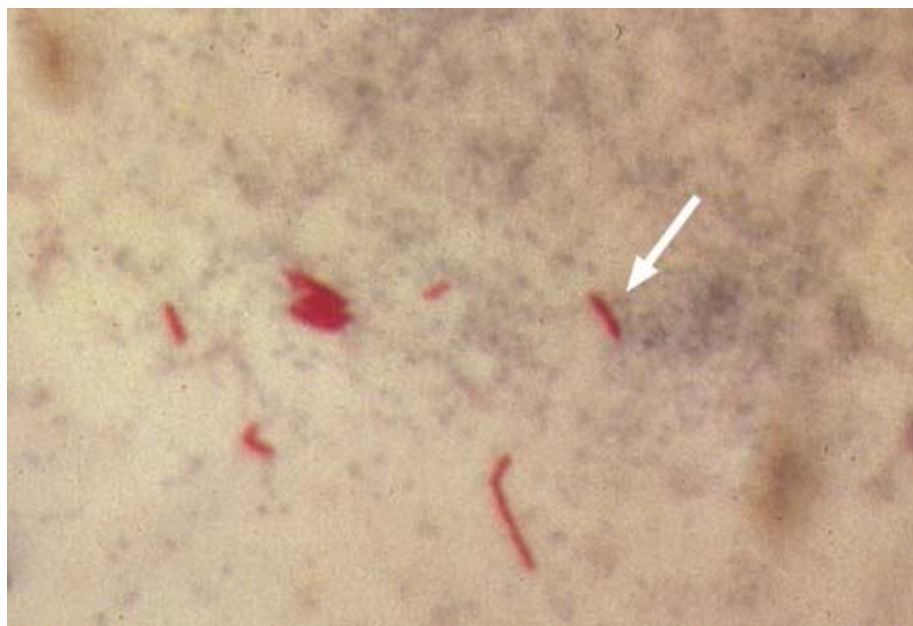


Figure 9. The microscopic view of acid fast bacilli stained *mycobacterium tuberculosis*

Cultivation and Identification

For the identification of tubercle bacilli, culture is considered as the reference technique. But; culture of *Mycobacterium tuberculosis* is expensive, slow and laborious. However, not more than 50% of clinically diagnosed patients are confirmed by culture. Conventional culture on LJ egg medium acquires two to six weeks. Therefore; looking for some other media for tubercle bacilli culture, i.e. 7H10 and 7H11 agar resulted in a higher and faster rate of identification. These media are more costly and usually used in advanced countries of the world. The radiometric respirometry technique (BACTEC) can drastically reduce the time needed for detection and more sensitive. Moreover; the median time being around 4 weeks both for sensitivity testing and detection. But; because of its specificity, imitations in terms of cost and biohazards; it is not appropriate for third world countries. Therefore; this method is usually used in the United States (Jain, 1996).

Automated Detection System

Radiometric BACTEC Method, Microbial Growth Indicator Tube (MGIT) and Detection of Microbial Antigen are used to diagnose the disease as automated detection system.

Genodiagnosis

For Multidrug-resistant *Mycobacterium tuberculosis*, the demand of an urgent detection is rapidly growing from clinician's side. They need exact molecular and rapidly diagnose. The involvement of various deletion, missense mutations and insertion within hypervariable region of *rpoB*, the gene encoding the β -subunit of the DNA dependent RNA polymerase of *Mycobacterium tuberculosis* to investigate the development of resistance to rifampicin (Vitali, et al., 1999). Description of the molecular level mechanisms of antituberculous drug resistance has contributed in the application and development of different PCR-based strategies designed for rapid detection of mutations linked with respective drug's resistance. The molecular assays are potentially more sensitive and rapid methods for drug resistance detection. They are hypothetically able to offer a similar day diagnostic report of clinical isolate. The usefulness of these tests is

dependent on their capability to identify all ordinary mutations related to drug resistance. These methods are summarized in table 3 and include SSCP analysis, direct sequencing of PCR products, dideoxy fingerprinting, heteroduplex analysis, a reverse hybridization-based line probe assay, base pair-mismatch assay, an RNA/RNA duplex, a rRNA/DNA bioluminescence labeled probe method, a luciferase mycobacteriophage strategy and other strategies (Heep M, et al., 2001). Amongst the so many procedures used to identify resistance against drugs associated with mutations i.e. automated DNA sequencing of PCR products is the mainly applied. Recently, the new technique the molecular beacons assay is described. This is preferably suited for genotypic examination, because it is capable to distinguish PCR products or amplicons as be synthesized in real time PCR. That may also differentiate DNA sequences that are different from one another by as small as a single nucleotide substitution. Rifampicin resistances can take place during the mutations in *rpoB* gene that encodes the β -subunit of RNA polymerase (Jin and Gross, 1988). There also $\geq 95\%$ of these mutations happen on an 81-bp fragment of the gene between bases 1276 and 1356 (432-458 in the *rpoB* gene of *Mycobacterium tuberculosis* and codon 507-534 in the *Escherichia coli rpoB* gene) (Miller LP, et al., 1994). This region is referred as "hotspot" or RRDR or rifampicin resistance determining region. It is marked as a target for commercial line probe assays and direct sequencing.

DNA probes

Developments of molecular genetics of mycobacterium have made it possible to recognize specific DNA sequences that are particular for individual mycobacterium species. These distinctive DNA sequences can be identified by means of labeled oligonucleotides, which are accurately complementary to the nucleotide sequence in the Mycobacterium genomic DNA. These DNA probes can recognize genus with species specific bacterial DNA sequences or high specificity. While; such probes have revealed the great specificity and sensitive, when utilized in research laboratories, these may some lose sensitivity and specificity if clinical samples used directly (Jain, 1996). *Mycobacterium tuberculosis* isolates can be tested with Inno-LiPA (Innogenetics Belgium), a line probe resistance determining hybridization assay, as explained in the manufacturer's directions. With biotinylated primers, an 81-bp region of the *rpoB* gene is amplified, that produce a biotinylated target sequence, and hybridized with specific oligonucleotide probes immobilized on a parallel strip. Following hybridization, alkaline phosphatase used to label the streptavidin was utilized to identify any hybrids. Inno-LiPA having 10 oligonucleotide probes; 19-23 bases in length. That surrounding the RRDR or 81-bp region of the *rpoB* gene. The assay's sensitivity may be enhanced if used on fresh samples. The mycobacterium can be damaged by deep freezing, causing the release of DNA that can be washed away.

PCR

Presently; a sufficient attention is given to the usage of polymerase chain reaction (PCR). The principle of the PCR technique is based on the amplification of a given DNA sequence to a large number of copies that can be identified by separation on gel electrophoresis and, subsequently, either with or without probing with a labeled oligonucleotide specific for the amplified DNA fragment. Approximately; all PCR laboratories are facing the contamination problem, because there are millions of suitable templates contain in product of the reaction that can be carried back to the next assay on pipettes, finger tips, in aerosols or clothing. The extraction of DNA from the cells of mycobacterium is another difficulty. This is one of the significant restraining factors in evaluating the sensitivity of PCR test for *Mycobacterium tuberculosis* (Jain, 1996). The extracted DNA from cells harvested from LJ slopes. A fragment of 270-bp is amplified with primers *rpoB*5' (5'-

TACGGCGTTTCGATGAACC-3') and rpob4 (5'-CCGCAGACGTTGATCAACA- 3') from every one of the clinical isolates and sequenced by means of these primers. Sequence investigation recognized diverse mutations affecting codons within the gene. A 183-bp amplicon amplified with primers rpob2 (5'- GGGTTTCGATCGGGCACAT-3') and rpob1 (5'- AGGAGTTCTTCGGCACCAG-3') in a reaction mixture containing 1× PCR buffer (50mM KCl, 20mM Tris-HCl [pH 8.4] and 2mM magnesium chloride), a 200nM concentration of each primer, a 200µM concentration of each deoxynucleoside triphosphate, and 1U of Taq DNA polymerase. After a preliminary denaturation step of 5 minutes at 95°C, PCR cycling executed for 30 cycles at 95°C temperature for 30 seconds, 60°C temperature for 30 seconds, and 72°C temperature for 1 minute. The 3 biprobes investigated in a 2nd round, asymmetric PCR on the LightCycler using strains representing the dissimilar mutations and a rifampin sensitive clinical specimen. An asymmetric PCR performed to preferentially amplify the DNA strand 2nd round, to which the biprobe can bind. PCRs (10µl) contained 1µl of the symmetric PCR product, MgCl₂ (5 mM), bovine serum albumin (1 mg/ml), Tris-HCl (pH 8.3) (500 mM), deoxynucleoside triphosphates (a 200 µM concentration of each) (Gibco BRL), rpoB reverse primer (5'-G GCACGCTCGCGTGACA-3') (5 pmol/µl), Platinum Taq polymerase (0.4 U) (Gibco BRL), a biprobe (5 pmol/µl) and Sybr Green I (Bio/Gene Ltd.) diluted 0.01%. The cycling strictures used denaturation at 95°C temperature for 20 seconds and 40 amplification cycles (temperature transition rate of 20°C/s) at 95°C temperature for 2 seconds, 55°C temperature for 1 second, 60°C temperature for 15 seconds, and 74°C temperature for 10 seconds. PCR cycling followed by melting curve analysis at 40 to 99°C (temperature transition rate of 0.2°C/s) with continuous fluorescence readings. Biprobe A (5' Cy5- CACCCAGCTGAGCCAATTC-biotin 3') spanned the mutations at codon 511. 69°C temperature is the determined melt temperature of the normal sequence, a mutation present the melt temperature of 65°C temperature. Biprobe B (5' Cy5- TTCATGGACCAGAACAACCC-biotin 3') spanned codon 516, and when a mutation present at codon 516, the melt temperature found to be 57°C temperature, compared to 64°C temperature in a wild-type sequence. Biprobe C (5' Cy5- GTTGACCCACAAGCGCC-biotin 3') spanned the mutations at position 526, and three peaks observed with this probe. The wild-type sequence melted at 61°C temperature, the sequence containing a mutation at position 526 melted at 49°C temperature, and a third melting peak which corresponded to a mutation at codon 531 observed at 66°C temperature. This third melting peak confirmed by testing additional strains identified to have this mutation and consistently reproduced (Edward, et al., 2001).

PCR-SSCP

To screen the gene mutations associated with resistance, PCR-SSCP and sequencing techniques were used. To confirm the identity of DNA fingerprint, an analysis made on sequential isolates. While; the treatment had a deep effect on modifications in drug resistance blueprints, the MIC for a specific agent remained constant in follow up isolates. Fingerprinting and mutational analysis of DNA demonstrated that the genome of MDR strains of *Mycobacterium tuberculosis* is comparatively stable throughout the way of treatment. The rpoB gene was the mainly mutated structural gene concerned a novel C to T mutation upstream of open reading frame (ORF)1 of the inhA operon was 51 detected and in drug resistance. There were no clues established for the existence of strain W in New York in this group of MDR strains (Victor, et al., 1997).

Table 5. Sequences of 6 sets of primers designed to amplify 6 overlapping fragments of the *rpoB* gene from *Mycobacterium Tuberculosis* isolates

Fragment	Amplicon size	Primer position	Primers
1	697 bp	-135 to -112* 541-562	Forward 5' GTT TAG TTG CGT GCG TGC Reverse 5' CTTGTCAAT GGT CTC GTC GAA
2	688 bp	451-472 1119-1139	Forward 5' TTC CCG ATG ATG ACC GAG AAG Reverse 5' GGA TCA GCT CGC CGA CCG TA
3	706 bp	1029-1048 1714-1735	Forward 5' CGA GGG TCA GAC CAC GAT G Reverse 5' GCG GGG CGA GAC GTC CAT GTA
4	681 bp	1624-1645 2284-2305	Forward 5' ATC GAT GCG GAC GGT CGC TTC Reverse 5' CGG GAT GTC GCG GGT GAT CTC
5	811 bp	2194-2215 2887-3005	Forward 5' TCC AAC CGC CTG GTC GAA GAG Reverse 5' CGT CGA CAC AAT GGC GTT
6	847 bp	2797-2815 +113 to +135*	Forward 5' TGT GCC CAC AGC GGC TGG Reverse 5' CTT TTT GAC CTC GCC ATA GGA C
*Denotes a primer position in the sequence before the start (-) or after the end (+) of the <i>rpoB</i> gene sequence.			

PCR Amplification

The conventional methods can be used for chromosomal DNA extraction (Orita, et al., 1989). A 157-bp fragment of the *rpoB* gene amplified by PCR with primers Tb8 (5' TGCACGTCGCGGACCTCCA3') and Tb9 (5'TCGCCGCGATCAAGGAGT3'). PCR carried out in 50 L of a reaction mixture containing 10 mM Tris (pH 8.0), 50 mM KCl, 100 M of deoxynucleoside triphosphates (dNTPs), 1.5 mM MgCl₂, 10 pmoles of each set of primers, 10 ng of chromosomal DNA and 1U Taq polymerase. The specimens then subjected to one cycle at 94°C temperature for 5 minutes, followed by 40 cycles at 94°C temperature for 1 minute, 55°C temperature for 1 minute, 72°C temperature for 1 minute, and a final cycle at 72°C temperature for 8 minutes to complete the elongation of the PCR intermediate products. Then; 2% agarose gels used to run on PCR products and examined for the presence of the 157-bp band after ethidium bromide staining (Miriam, et al., 2001).

Comparison, reading and Interpretation of data

IS6110 DNA genotyping molecular studies and fingerprint analysis of DNA, based on IS6110 Southern blot hybridization pattern, executed by using a standardized procedure (Van, et al., 1993) can be compared on a Sun Sparc Workstation (Sun Micro Systems, Santa Clara, CA), using Bio Image Whole Band Analyzer software version 3.4 (Bio Image, Ann Arbor, MI) (Sonal S. et al., 1997).

Electropherogram view

Four coloured peaks corresponding to the four labelled letters comprising DNA nucleotide (Figure 3). Base calling view: Where the nucleotide sequence appeared in the form of letters (A, T, C, G). (Nagwa Khamis, et al., 2004).

Statistical Analysis

Sensitivity and specificity of the PCR-SSCP method can be determined by using the test for 2X2 contingency tables. Differences in the mean MIC logs among strains with specific mutations can be calculated by the two-sample Wilcoxon rank-sum test (Mann-Whitney U test) (Miriam, et al., 2001).

Serodiagnosis

The ELISA and IID one step tuberculosis tests can be performed for diagnostic purpose.

ELISA test

Like all other bacteria, the tubercle bacillus, is rich in antigens that stimulate antibody production. A number of tests are established in the last one century to identify particular antibody response in supposed patients. These assays are used either for fragments of AFB or whole bacteria, purified antigens both by chromatography, culture filtrates, partially purified antigens and by DNA recombinant technology. Different methods are used to carry out the assays, i.e radio-immuno-assay, immunoblot and ELISA. Furthermore, instead of looking for particular antibodies, efforts have been made to identify antigens in clinical specimens using particularly monoclonal and polyclonal antibodies. There are a number of inherent complexities in hunting for antibodies to diagnose tuberculosis. Response of antibodies may persist for years specifically if an individual immune system identified a dormant bacilli population. While; in elevated occurrence region, it may be hard to differentiate existing from old illness. But, currently, no test is suitable for the tuberculosis diagnosis. Many physicians use the tuberculin test for the diagnosis purpose. It is not a diagnostic test as it disclosed only whether a person is infected or not with *Mycobacterium tuberculosis*. However; can't distinguish between disease and infection. In countries with high endemic of tuberculosis, this assay is of no use at all, because a big part of the population is already infected with the bacilli (Jain, 1996).

International immune diagnostic (IID) one step tuberculosis test

The serodiagnostics test is limitedly used in research and investigation. It is not yet trust worthy and may be developed in future for rapid tuberculosis diagnosis. 2.8.10 Gas Liquid Chromatography and HPLC It is used to detect the specific biomaterial of MTB in given patients specimen. It also detect the AFB fragments culture filtrates, partially purified antigen/ purified antigen of MTB to produce a conclusive clinical report. It is the chromatographic elucidation of mycobacterium or its metabolites for diagnostic or research purpose.

Susceptibility Evaluation

In the treatment of difficult tuberculosis cases, the role of drug sensitivity testing should not be underestimated. Hence; this is an additional significant area where the laboratory has significant function. Antituberculosis drug therapy depends on the susceptibility of the tubercle bacilli. Drug resistance is the potential of tuberculosis strains to grow and survive even if exposed to optimum drug concentrations that kill or inhibit the parental bacilli, and shift this trait to its progeny. Drug susceptibility testing should be preferred for the individuals as under;

- 1 Patients that remain smear (sputum) positive even after 2-3months' of intensive therapy
- 2 Interruption or treatment failure cases

- 3 Patients having tuberculosis symptoms and are in close contacts of MDR tuberculosis cases
- 4 Patient having the symptoms of tuberculosis and are directly with tuberculosis patients e.i. prisoners, laboratory workers health and care workers (Karin , et al., 1997).

The quality of laboratory susceptibility testing has impacts directly on tuberculosis treatment and is of vital significance. Reporting and laboratory methodology must be calibrated, standardized and controlled appropriately. All drugs should be tested at its critical concentration or the concentration that inhibits growth of the majority of wild strains of *Mycobacterium tuberculosis* without markedly affecting the growth of resistant mutants.

Table 6. Critical drug concentrations for routine susceptibility testing (µ/ml)

Drug	Radiometric	Conventional	
		Middlebrook 7H10	Löwenstein-Jensen
Isoniazid	0.1	0.2	0.2
Rifampicin	2.0	1.0	40.0
Ethambutol	2.5	5.0	2.0
Streptomycin	2.0	2.0	4.0

(Karin, et al., 1997).

Critical drug concentration can be defined as a sufficient degree of drug to make it rationally sure that the concerned strain is unlike from a tubercle bacilli sample that have not at all come in contact with the drug. Currently; the known mechanism drug resistance acquisition of *Mycobacterium tuberculosis* is spontaneous mutation (Jain, 1996). There were various techniques; E test, bioluminescence, polymerase chain reaction single strand conformational polymorphism (PCR-SSCP) etc used for drug susceptibility testing of tubercle bacilli in past. The proportion method that use Lowenstein Jensen (L-J) medium is most widely accepted.

Drug susceptibility

All clinical isolates of *Mycobacterium tuberculosis* used for susceptibility testin of antituberculous drugs rifampicin (RIF), isoniazide (INH), ethambutol (ETB), ciprofloxacin (CIP) and streptomycin (STM). The drug concentrations used in the proportion method on Middlebrook 7H11 agar medium and LJ medium as recommended by the Tuberculosis Researchers. The reference strain *Mycobacterium tuberculosis* H37Rv, sensitive to all the antituberculosis agents, can be used as the control strain in each test batch (NCCLS, 1994). While; the proportion methods use the Middlebrook 7H11 agar and LJ medium to executed experiments with accordance of the established standards or procedures (Sachdeva , et al., 2002).

Table 7. Miscellaneous MIC,s of anti-TB drugs against M. Tuberculosis.

#	Origin	RFP µg/ml	PZA µg/ml	ISN µg/ml	ETD µg/ml	CIP µg/ml	STP µg/ml
1	Dept. of Respiratory Medicines, Semmelwies University, Budapest, Hungary.	>32					
2	A.K. Praharaj et.al, Armed Forces Medical College, Pune, India.	>64	>100	>1	8		
3	Memorias Do Insti. Oswaldo Cruz, NCCLS, 2002	40		0.2-1	2		4
4	Dept. of Clinical Microbiology, Christian Medical College, Vellore, India.	64		0.4			
5	American Society of Microbiology J of Clinical Microbiology. (Middle Brook 7H10, BACTEC 460)					≤0.5	
6	Pakistan Medical Research Centre for Tuberculosis & Chest Diseases, KEMC/ Mayo Hospital, Lahore, Pakistan.	40	100	0.2	2		4
7	Arneimittelforschung, Germany						
8	WHO/CDS/TB/2001.288						
9	CDC/, USA, 2004						
7	Resistant to higher INH concentration	4-32					
8	Resistant to Ethambutol			0.1-1			2-8
9	Resistant to low INH concentration		100-		1-5		
	Fully susceptible	1-50	400	0.1-	1-7.5		1-10
		5-50	1-400	1-10	1-5		1-10
	Richard J., JR Wallace et.al; 1986,	1-50	1-400	10-	1-5		1-10
		1-50	1-400	100			1-10
	Ahmed Yelmiaz et.al; 2004 LJ			1-100	0.5-		
10	Public Health Agency, Canada, (BMM)	0.125-16	0.25-32		64		0.5-64
11		40	0.2-1		2		4
12		0.25-16	3.2-0.05		0.5-32		0.5-32

E test susceptibility

Susceptibility by E test method (AB Biodisk, Sweden) performed on Middlebrook 7H11 agar with Oleic acid-albumindextrose- catalase (OADC) supplement. Bacterial suspension made in sterile distilled water and matched with MacFarland No.3. The surface of the agar swabbed with the suspension (Kakkar N, et al., 2000). The plate's pre incubated at 37°C in CO₂ jars for 24 h. E test strips placed on the surface of the agar, the plates sealed with cling films and again incubated at 37°C in CO₂ jar for 7-10 days. E test strips containing gradient concentrations of the INH, RIF, EMB, STM and CIP used. Minimum inhibitory concentrations (MICs) recorded and reported as sensitive or resistant as per AB Biodisk manufacturer's guidelines (Hoffner SE, et al., 1994)

TREATMENT OF TUBERCULOSIS

The tuberculosis is treated by certain drugs (chemotherapy), radiation and surgical methods. The detail of all treatment strategies as given below,

Chemotherapy of Tuberculosis

There are four major 1st line drugs – rifampicin, isoniazid, pyrazinamide and ethambutol available to cure the tuberculosis. There also 2 nd line drugs available to treat the resistant mycobacterium tuberculosis.

1st line therapy of susceptible *Mycobacterium tuberculosis* (MTB)

There are toxicity induction, variance in dose and regimen, poor supervision, clinical complication and resistance developments, are the major hurdles in the chemotherapy of susceptible and resistant mycobacterium. The first line therapy comprised of four or some time five drugs – rifampicin, isoniazid, pyrazinamide, ethambutol and streptomycin. The dose, protocol and regimen are decided on basis of severity & type of disease and pathological & personnel history. The clinical record of body temperature, weight, cough and general well being is more important than many sophisticated investigations to assess the success of retreatment regimens. During the initial therapy, bacterial count in sputum specimen should be done every week to assess mycobacterial burden. It should be followed by sputum smear examination for AFB at monthly intervals till sputum conversion. Development in the results of bacteriological assays of sputum is the specific marker of the success of a chemotherapy regimen. Sputum culture for mycobacterium should be done every three months till the end of the therapy. Improvement assessed by X-ray is a non-specific and less reliable parameter and should be done every third month. (Herendra & Shah, 1998). Directly observed treatment all over the chemotherapy course is important with objective of 18-24 months of therapy. Moreover; an initial 4 months of intensive therapy should be incorporated to make sure the successful eradication of disease. The continuation period may be reduced if 12 months of therapy is provided subsequent to sputum conversion as confirmed by 3 successive monthly negative cultures. Confirmation of HIV status is clinically importance, because HIV sero-positive patients can demonstrate augmented adverse effects from antituberculosis medications. While; the adverse drugs actions are not potentially life threatening. All effort should be completed to train patients through psychological support and palliation. Medicines with identified side effects can be given in divided regimens to enhance the tolerance. The tuberculosis patients having serious adverse effects should be treated in clinical setting or hospital.

There are so many patients successfully treated with DOT's ambulatory therapy provided to ensure eradication of tuberculosis illness. Therefore; preventing treatment interruption, enabling patients to remain in employment, release up scarce beds, decreasing costs and therefore. Thus; tuberculosis sick individuals are trained for fundamental infection control procedures: sputum disposal, separate sleeping place, safer coughing, sunlight and ventilation. It must be kept in mind that the individual has previously had contact over an elongated period of time with persons he/she lives with has extra risk for this disease. The patient should be admitted, if any of the following decisive factors are involved; 1) Previous treatment interrupter, 2) Poor clinical condition, 3) Major adverse drug reactions, 4) Complications ie haemoptysis, and 5) Poor social circumstances

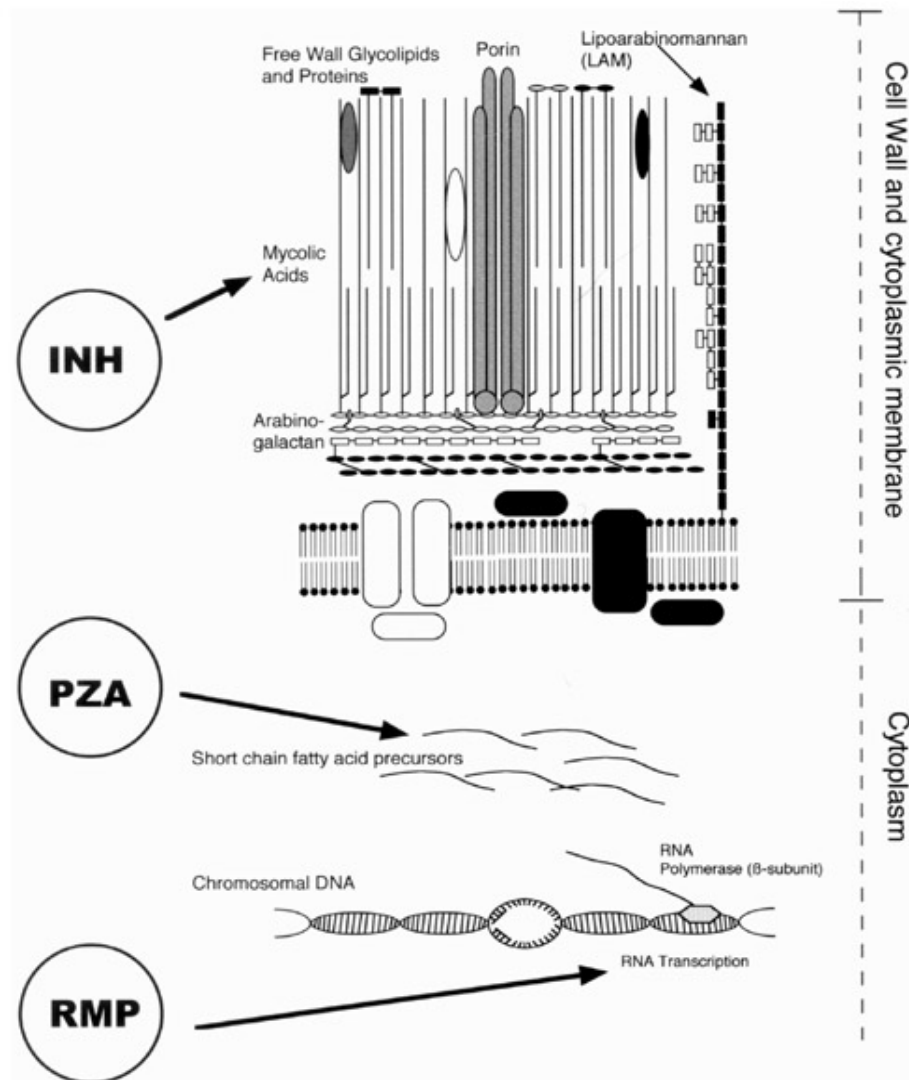


Figure 12. Molecular level mechanism of action of isoniazid, pyrazinamide and rifampicin.

Characterization of to manage the Multidrug Resistant tuberculosis patients can be done as by under;

- 1 Provision of a social worker for support and counsel,
- 2 Rationalizin the drug susceptibility examination of specimens from MDR tuberculosis patients
- 3 Keeping registers updated,
- 4 Provision of key nursing staff to provide direct and continuity observation of therapy,
- 5 Therapeutical drug compliance monitoring,
- 6 Improving the motivation and education of patients,

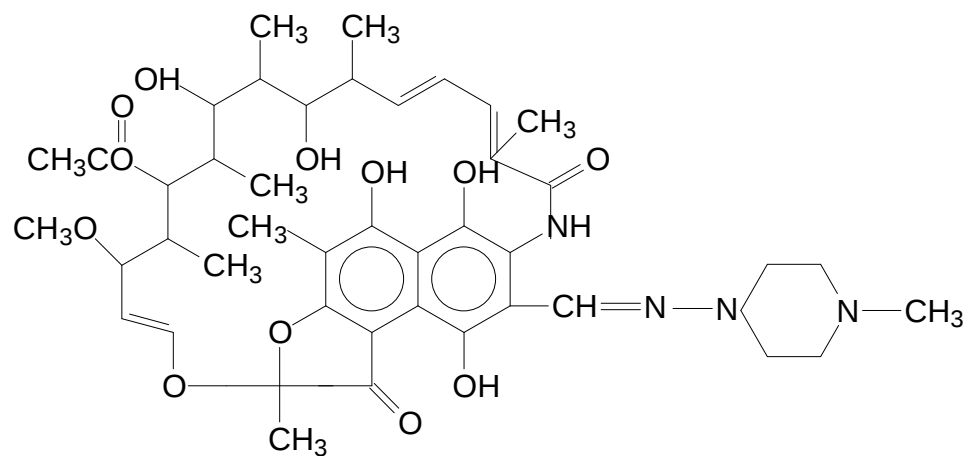
- 7 Developing measures for rapid recall if patients interrupt their therapies,
- 8 Evaluating and tracing contacts rapidly (Karin, et al., 1997).

Table 8: Recommended duration of therapy of tuberculosis

No.	Regimen	Months
1	Isoniazid, Rifampicin, pyrazinamide	6
2	Isoniazid, Rifampicin	9
3	Rifampicin, Ethambutol, Pyrazinamide	6
4	Rifampicin, Ethambutol	12
5	Isoniazid, Ethambutol	18
6	Others	24

Pharmacological of Rifampicin.

It having broader antimicrobial activity than isoniazid and isolated from the mold streptomyces soil. Rifampicin has been found application in the treatment of number of different bacterial infections. Since resistant strains quickly emerge during treatment it is never recommend as a single agent in the threpy of active patients of tuberculosis.



Structure of Rifampicin

Figure 13. Molecular structure of Rifampicin.

Mechanism

Rifampicin inhibits the transcription by interacting with the β subunit of bacterial (rather than human) DNA dependent RNA polymerase. Rifampicin is specific for prokaryotes and inhibits RNA synthesis by suppressing the initiation step. Resistance: Resistance to rifampicin can be caused by a mutation in the affinity of the bacterial DNA polymerase for the drug or by decreased permeability. Pharmacokinetics:

Rifampicin is moderately absorbed after oral administration. Distribution of rifampicin occurs to all body fluids and organs. Adequate levels are attained in the CSF even in absence of inflammation. The drug is taken up by the liver and undergoes enterohepatic cycling. Rifampicin itself can induce the hepatic mixed-function oxidases, leading to a shortened half life. Elimination of metabolites and the parent drug is via the bile into the feces or via the urine. There is orange-red coloration of urine and feces and tear may permanently stain the soft contact lens. Adverse effects: Rifampicin can produce the nausea, vomiting, rashes and fever. There is jaundice introduced in the chronic liver disease, alcoholic and elderly patients. Interaction: Rifampicin induces a number of cytochrome P450 enzymes as mentioned in figure. It can decrease the half lives of drugs that are co-administered and metabolized through this system.

Pharmacological of isoniazid

The hydrazide of isonicotinic acid or isoniazide is a synthetic analog of pyridoxine. This drug is not at all given as single therapeutic agent in the treatment of active tuberculosis. It is also the most effective of the antituberculosis drugs. Isoniazide has revolutionized the successful eradication of tuberculosis.

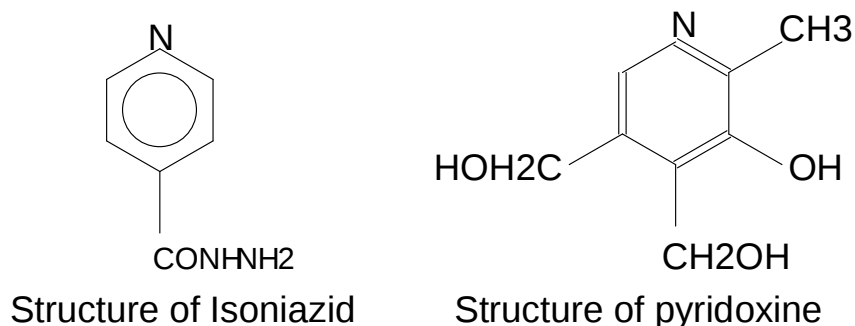


Figure 16. Molecular structure of Isoniazid and Pyridoxine.

Mechanism: This pro-drug is activated by mycobacterial catalase-peroxidase (KatG). The biochemical and Genetic evidence has implicated at least two dissimilar target enzyme concerned in the synthesis of mycolic acid. Mycolic acid is a unique class of very long chain, β -hydroxylated fatty acid found in mycobacterial cell wall. Decreased mycolic acid synthesis corresponds with the loss of acid fastness after exposure to isoniazid. The targeted enzymes are enoyl β -ketoacyl-ACP synthase (KasA) and acyl carrier protein reductase (InhA). The activated drug covalently binds to and inhibits these enzymes, which are essential for the synthesis of mycolic acid. Resistance:

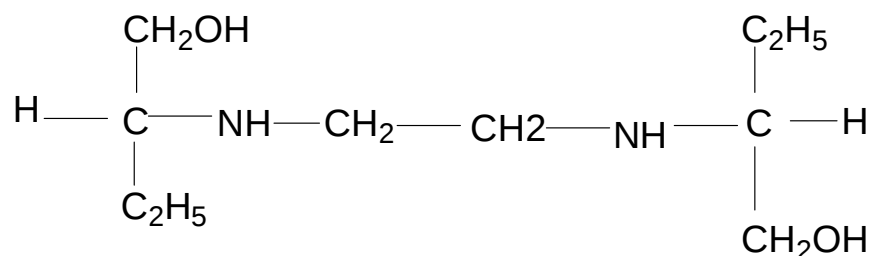
This is associated with several different chromosomal mutations, each of which results in one of the following; mutation or deletion of KatG (producing mutation incapable of prodrug activation), varying mutation of the acyl carrier proteins, or overexpression of InhA. Cross-resistance does not occur between isoniazid and other antitubercular drugs. Pharmacokinetics: Isoniazid undergoes hepatic N-acetylation that is regulated genetically, with the fast acetylator trait being autosomally dominant. Chronic liver disease decreases metabolism, and dose must be reduced. Excretion is through glomerular filtration, predominantly as metabolites. Slow acetylators excrete more of the parent compound. Severely depressed renal function result in accumulation of the drug, primarily in slow acetylators.

Adverse effects: The adverse effects are fairly low except hypersensitivity. The adverse effects are related to dosage and duration of administration.

Peripheral neuritis: Manifested as paresthenia which is the most common adverse effect, appears to be due to a relative pyridoxine deficiency. It is corrected by pyridoxine (vitamin B6) supplementation and therefore particularly recommended to lactating women to intake vitamin supplementation. Hepatitis and idiosyncratic hepatotoxicity: potentially fatal hepatitis is the most severe adverse effect. That is caused by a toxic metabolite of monoacetylhydrazine, formed during the metabolism of isoniazid. Incidences are further aggravated in aged, rifampicin taking and alcohol drinking patients. Other adverse effects: Mental abnormality, convulsion in patient prone to seizures, optic neuritis and hypersensitivity reactions including rashes and fever. Interactions: The drugs that interfere the P-450 hepatic microsomal isozyme i.e. Phenytoin may potentiate the unwanted effects profile.

Pharmacological of Ethambutol

This drug is bacteriostatic and specific for most of *M. tuberculosis* and *M. kansasii*. Ethambutol inhibits arabinosyl transferase - an enzyme that is important for the synthesis of the mycobacterial arabinogalactan cell wall. While; the resistance is not a serious problem if the drug is employed with other antitubercular agents. Ethambutol can be used in combination with pyrazinamide, isoniazid and rifampicin to treat tuberculosis.



Structure of Ethambutol

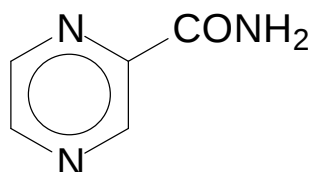
Figure 20. Molecular structure of Ethambutol

Absorbed on oral administration ethambutol is well distributed throughout the body. Diffusion into the central nervous system is therapeutically sufficient in meningitis tuberculous. Both; the metabolites and parent drug are excreted by tubular secretion and glomerular filtration. Optic neuritis is the most significant adverse effects, which results in diminished visual acuity and ability

to differentiate between green and red lost. Visual acuity should be periodically examined. Discontinuation of the drug results in reversal of the toxic symptoms. In addition, urate excretion is decreased by drug; thus gout may be exacerbated as mentioned in figure. Antitubercular have a therapeutic margin, as with any drug, is difference between the maximum concentration that can be given without provoking drug toxicity and minimum drug concentration required to inhibit the growth of *Mycobacterium tuberculosis*. This therapeutic margin varies for the various 1st line medicines (Richard, et al., 2006).

Pharmacological of Pyrazinamide

Pyrazinamide is a synthetic, orally effective, bactericidal antitubercular agent used in combination with isoniazid and rifampicin. It is bactericidal to actively dividing organisms, but the mechanism of its action is unclear. Pyrazinamide have to be enzymatically hydrolyzed to pyrazinoic acid, that is the active drug form of the Pyrazinamide. Some resistant strains lack the pyrazinamidase. Pyrazinamide is active against tubercle bacilli in the acidic environment of lysosomes as well as in macrophages.



Structure of Pyrazinamide

Figure 22. Molecular structure of Pyrazinamide

Pyrazinamide distributes all through the body, penetrating the CSF. It undergoes widespread metabolism. About one to five percent of patients taking 65 isoniazid, rifampicin and pyrazinamide may experience liver dysfunction. Urate retention can also occur and may precipitate a gouty attack.

Table 9: Some characteristics of first line drugs used in treating tuberculosis

Drug	Adverse effects	Comments
Ethambutol	Optic neuritis with blurred vision, red-green color blindness.	Establish baseline visual acuity and color vision, test monthly.
Isoniazid	Hepatic enzyme elevation, hepatitis, peripheral neuropathy.	Take baseline hepatic enzyme easements, repeat if abnormal or patient i9s at risk or symptomatic clinically significant interaction with Phenytoin and antifungal agents. (azoles).
Pyrazinamide	Nausea, hepatitis, hyperuricemia, rashes, joint ache, gout (rare) .	Take baseline hepatic enzyme and uric acid measurements. Repeat it abnormal or patient is at risk or symptomatic.
Rifampicin	Hepatitis, GI upset, rash, flu like syndrome, significant interaction with several drugs.	Take baseline hepatic enzyme measurements an CBC count repeat if abnormal or patient is at risk or symptomatic. Warn patient that urine and tears may turn red-orange in colour.

Second line therapy of Resistant MTB

The fundamental principles of therapy of multi drug resistant tuberculosis are as under;

- 1 Use of 1st line drugs should be preferred since they are less toxic and more effective
- 2 Drugs chosen for the therapy should not be used in past or used regularly for shorter time,
- 3 Medicines are to be given in enough doses and for sufficient period of time (Herendra & Shah, 1998).

Directly observed therapy throughout the intensive phase of therapy is should be considered as national policy. Good adherence throughout the intensive treatment phase, that reduces the time of the total bacterial load in the patient. This also is critical to the prevention of Multidrug resistance tuberculosis. This is particularly factual for sputum smear positive patients having higher bacterial load. During the follow up phase; DOT is significant to facilitate stop reversion. The continuous supply of tuberculosis medicines to cure points is critical in the prevention of resistance against drugs (Karin, et al., 1997). The resistant strains of *Mycobacterium tuberculosis* against a particular agent emerge during treatment with single drugs. Therefore multidrug therapy is employed when treating tuberculosis in an attempt to prevent or delay the appearance of resistant strains. Management of patients, whose treatment with commonly used antitubercular drugs has failed, is problematic and so one has to depend on less potent, potentially more toxic and costly drugs in various combinations. The prohibitive cost and nonavailability of drugs further deteriorate the situation. Moreover, it is advantageous to use four or more medicine for such patients for a minimum of two years. Drug resistance does not give any selective benefit to the bacterium unless it is exposed to that drug. Therefore; the drug-resistant mutants flourish and the sensitive strains are killed under such circumstances. On exposed to a 2nd course of drug treatment, the patient may eventually gain the bacilli resistant against two or more drugs and mutants resistance developed in *Mycobacterium tuberculosis* strains. Thus; the serial selection of resistance drugs is the major mechanism for the MDR strains development. The patients with MDR strains comprised of a pool of chronic infections. That proliferate main MDR resistance. In addition to the accumulation of mutations in the individual drug target genes, the permeability hurdle imposed by the *Mycobacterium tuberculosis* cell wall can participate contribute to low level drug resistance the development. Patients should be educated to report immediately if they feel any loss of hearing, ototoxicity with prolonged usage of aminoglycosides.. Moreover; the clinical development should be documented regularly and obtained a chest radiograph after every three months (Karin, et al., 1997). This is also significant that nurses and clinicians create enough use of resources. The investigations and ordering of costly medicines in an unsystematic way leaves smaller amount of resources available for more imperative interventions i.e. tracing patients who have missed therapy arrangements. To complete the full course of prescribed medication, adherence to patient's management is quite important. This frequently depends on accessibility of the service, satisfactory counseling, ongoing support of health care staff and the attitudes.

Second Line treatment protocol

Treatment of drug resistance in pulmonary tuberculosis is a great challenge for treating physicians. INH should be used in all the anti-tubercular regimens as it is one of the highly effective drugs with least toxicity and also costs less. Cross resistant drugs should be avoided as they are not effective and increase toxicity. Drugs with one-way cross resistance Viomycin and Capreomycin Viomycin and Kanamycin Kanamycin and Streptomycin Thiocetazone and Para-aminosalicylic acid. Preferably three new drugs should be given along with first line drugs. If second line or newer drugs are prescribed, then at least 4 or 5 drugs to be

given. Addition of a single drug in failing regimen is contra- indicated. Considering the toxicity of reserve drugs, doses should be increased in time with optimal tolerance of the patient to achieve best results. Retreatment should always be given preferably in hospital under strict supervision for close observation of adverse drug reactions commonly associated with the reserve drugs. Intermittent chemotherapy is normally not effective and should not be given in multi drug resistant tuberculosis. An early surgical interference can be planned if disease is limited and patient's general condition permits (Herendra & Shah, 1998).

Table 10: Approach 1 for the treatment of MDR tuberculosis Intensive phase: 4 months

Drug	Daily dosage	
	Average (mg/kg)	Maximum (mg)
Kanamycin	15	1 000
Ethionamide	10-20	1000
Pyrazinamide	20-30	1 600
Ofloxacin or Ciprofloxacin	7.5-15	800
	7.5-15	1500
Ethambutol or Cycloserine	15-25	1200
	10-20	1000

Table 11: Continuation phase: 12-18 months

Drug	Daily dosage	
	Average (mg/kg)	Maximum (mg)
Ethionamide	5-10	750
Ofloxacin or Ciprofloxacin	7.5-15	800
	7.5-15	1500
Ethambutol or Cycloserine	15-25	1200
	10-20	1000

(Karin et al, 1998)

The standard regimen for treatment of Multidrug Resistant tuberculosis cases consists of a four month intensive phase with ethionamide, kanamycin, ofloxacin, pyrazinamide, and ethambutol or cycloserine (five drugs), followed by a 12-18 month continuance phase with three drugs (ofloxacin, ethionamide and cycloserine or ethambutol). The medicines should be given five times a week in clinics and seven times a week in hospitals settings. The period of continuation can be reduced to 12 months of thereapy is be given after sputum conversion as confirmed by three successive monthly negative cultures.

Newer drugs

Effective new regimens have been developed by using newer drugs in combination with other ones. The new drug delivery system and rapid strides in molecular biology and biotechnology will soon make their impact in the therapy of multidrug resistant tuberculosis. These medicines are Rifampicin derivatives - Rifabutin/ Rifapentin (CGP 29861/CGP 7040), Quinolones - Ciprofloxacin, Ofloxacin, Pefloxacin, Lomfloxacin, Sparfloxacin, Clofazimine - dye- Sulphone derivatives, Macrolides - Roxithromycin/Clarithromycin/Azithromycin, 5. Betalactam Antibiotics with Clavulanic acid, Cephalosporins – Cefornide, Folate Antagonists - Trimethoprim derivative: Brodinoprim, Metioprim, Miscellaneous - CQQ (Gangamycin), Fusidic acid, Immunomodulators - Interferon Y, Interleukin -2, TNF.

Laser Therapy

Laser has been tried in some countries of the world to manage the tuberculosis. It is believed to be valuable in disease of multicavitary with profound bacterial load, particularly when there are improved alterations of medical treatment failure. Laser is supposed to be used in fast killing of bacteria. Thus; it reduces the bacterial load. Secondly, laser helps in early cavity closure and increase the incursion of antitubercular medicines in walled off lesions. Because of the tubercular granuloma, it has proven benefit in the bronchial and tracheal stenosis. A variety of types of laser have been applied in tuberculosis resistant case that undergoes surgical treatment. Therapeutic lasers (Helium-Neon, Surgical (CO₂ and YAG), Ultra Violet and semiconductive are used for this purpose. Thus; laser decreases the trauma of the surgery and volume to decrease the post operative complications and raise the effectiveness of surgical treatment.

Radiotherapy

Chest X-ray deterioration can be a sign of failure but deterioration may be because of one of the three reasons: 1) supervening carcinoma, 2) Pulmonary embolism, 3) Intercurrent pneumonia. After two to three weeks, a repeat chest X-ray may possibly demonstrate development in the case of pulmonary embolism or intercurrent pneumonia. Apparently; if radiological deterioration is not accompanied by bacteriological corrosion, is less likely to be contributed by tuberculosis.

Surgical Resection

In view of the frequent treatment failure in spite of the planned and supervised chemotherapy in multidrug resistant tuberculosis, the surgical interference is now increasingly used as an adjuvant to medical treatment. The main aim of surgery in patients with multidrug resistant tuberculosis is to decrease the bacillary load. Surgical treatment is mainly shown in cases with continual destroyed lobe or cavitation or lung in which medical therapy is likely to fail or increased the probability of reversion. Surgical treatment can be used when; (a) disease is limited to one anatomical area so that removal of the lung will not involve extensive lung resection. (b) Pulmonary function is adequate. (c) drug therapy should be continued after operation also. Before the advent of chemotherapy, surgical intervention was widely practiced. Eventually; it became evident that drug therapy alone was sufficient to treat most cases in the chemotherapy era. Moreover; it was emphasized that the MDR tuberculosis therapy is 1st and leading chemotherapeutic. However; there are four indications for surgery; 1) Be short of clinical and radiographic development after six months of enough treatment, 2) Determination of positive sputum cultures, 3) Lung/ gross lobe/ residual destruction and/ or cavitation, 4) Relapse in the similar place after a previous proper course of chemotherapy. The choice of to execute the extent of surgery or surgery i.e. pneumonectomy, lobectomy should be complete after anatomical localisation of tuberculosis by CT scan. Over and over again; lower lobe's apex is concerned mutually with a respective upper lobe

and the previous should also be detached. Scans Perfusion are helpful in determining how much lung's functioning is probable be removed. Fundamental spirometry i.e. FEV1 and FVC is enough in evaluating lung function in the greater part of tuberculosis cases. The electrocardiogram is helpful for not including pulmonary hypertension that would contraindicate in surgery.

Chemoprophylaxis

Those in contact with multidrug resistant tuberculosis patients are ideal candidates for Chemoprophylaxis. It is the only proved treatment normally recommended and efficacious for the avoidance of tuberculosis. But in contacts of INK resistant cases, Rifampicin can be used which has efficacy equal or even greater than INH as a chemo-prophylactic agent. In contacts of cases resistant to both Rifampicin and INH, Pyrazinamide (Z) + Ethambutol (E), Pyrazinamide with or without Ciprofloxacin/Ofloxacin can be given.

PREVENTION OF TUBERCULOSIS

Prevention of TB is a major issue of TB control programmes. The community and nosocomial transmission minimized by certain preventive measures.

Prevention of Transmission

The accepted guidelines for transmission prevention of tuberculosis in health care settings are as under;

- 1 The active tuberculosis patients must be recognized rapidly by using the most sensitive available laboratory methods.
- 2 Effective antitubercular therapy should be started urgently in diagnosed patients.
- 3 Cough inducing procedures e.g. sputum induction, bronchoscopy, administration of aerosol treatment, in suspected or confirmed tuberculosis cases should be started in isolated rooms.
- 4 Suspected or even confirmed infectious tuberculosis patients should be placed in isolation immediately.
- 5 Examination of drug resistance patterns on regular basis in the community.
- 6 Health care workers and patients exposed to multidrug resistant infectious tuberculosis case should be examined regularly for infection/disease.
- 7 The precautionary principle includes as under;
 - a. Receiving ongoing training and education of the consequences of MDR tuberculosis, disease transmission and tuberculosis pathogenesis.
 - b. Persistent awareness of risk situations and their avoidance should be stressing.
 - c. Informing about the raised risk of acquiring the tuberculosis and MDR illness may become HIV positive. Secret HIV assay and substitute employment are obtainable for HIV positive patients.
 - d. Worldwide infection prevention measures execution in all health care facilities, including secure waste clearance.
 - e. Severely controlling the coughing behavior and collecting sputum in especially containers.

The tuberculosis monitoring programme for elevated hazard surroundings should have a confidential disease monitoring file to screen methods for disease as well as other health associated recorded informations. The fundamentals of a tuberculosis monitoring programme are as under;

- 1 Employment baseline and profile screening
- 2 A standardized health questionnaire completed for every employee for purposes of recompense. That questionnaire should tell past tuberculosis disease,

- 3 Be encouraging to describe the first symptoms of tuberculosis disease i.e. weight loss, tiredness, loss of appetite and persistent cough. These indications be liable to come on gradually and may be approved off as stress or flu. Lots of the health personnel have refused to be examined until the tuberculosis has caused permanent damage of lungs.

Prevention of Susceptible/ MDR Tuberculosis

Two major approaches are used to stop multidrug resistance: (a) Identification of cases with infection of tubercular and their prophylactic treatment, (b) Identification and treatment of multidrug resistant tuberculosis patients. The aim is to identify their disease and to prevent further transmission. The objective is to avoid the 5-10% danger of following expansion of illness.

Vaccination

Bacillus Calmett Guirin (BCG) vaccination status and causal health conditions may enhance susceptibility of community to disease. While; previous contact with tuberculosis confirmed cases also help in sickness. A Mantoux tuberculin skin test (TST) and a baseline chest x-ray can be conducted to understand the exact situation.

Health Care Awareness/ Education

The danger of tuberculosis infection depends on exposure time, intensive exposure to this case and the severity of disease in the source case. Therefore, all Health Care Workers (HCWs) are not at equal danger of acquiring disease. For numerous HCWs cadres the hazard is approximately identical to that of the general population.

High risk: Health Care Workers are at high risk that are close contact with smear positive MDR tuberculosis patients or infectious cases i.e. other medical staff and nursing staff in tuberculosis centres and wards. HCWs involved in aerosol producing procedures i.e. respiratory technicians, pulmonary physicians and other medical staff involved in sputum induction, bronchoscopy, aerosolised pentamidine therapy, autopsy procedures and tracheal intubation.

Medium risk: Staff concerned in collection of sputum from tuberculosis suspected patients are in close contact for a prolonged period of time at medium risk.

Low risk: People concerned in management of tuberculosis patients, providing the facilities and supporting staff i.e. cleaners, administrative staff and porters.

RECOMMENDATIONS

Based upon the findings of the present study, the recommendations can be divided into following major categories:

General Recommendations for Tuberculosis

- Implementation of standard infection controlling procedures and guidelines to reduced the spread of tuberculosis pathogenicity in and outside the institutions/ hospitals. ▶ It is highly required to introduce a national mycobacterial infection controlling and molecular resistance surveillance programme that is not currently exist in Pakistan. The computer technology and medical informatics may be used for accurate data collection, efficient characterization and timely analysis to successfully overcome the global tuberculosis burden.
- The systematic surveillance, quality assurance and digital data support needed for decision making regarding the chemotherapy and/ or prophylaxis purpose of susceptible and resistant *Mycobacterium tuberculosis*. It will be forthcoming to predict emerging resistance among available anti-tubercular drugs, leading to effective treatment that could control dissemination of *Mycobacterium* resistance.
- Understanding the physiology/ morphology, ecology, genetic and molecular basis of resistance of *Mycobacterium* TB should be increased for successful control of this dangerous microbe.
- Strengthening the curriculum of human health care professionals in the area of disinfection, house keeping, sterilization and factors contributing to its spread, including inappropriate treatment protocols with ineffective regimens. ▶ Involving the drugs experts (particularly the qualified pharmacists), governmental agencies and welfare organizations to assure the quality medication, therapeutical modifications and implementing the , prophylaxis procedures against tuberculosis.
- Establishing the laboratory standards for efficient and accurate detection of molecular and genetic basis of resistance of *Mycobacterium tuberculosis*. These standards include sample collection, transportation, physical examination, molecular identification, genetic characterization and reporting techniques used for molecular epidemiology.

Recommendations to improving the awareness against TB

- Educating the patients regarding appropriate use of 1stline drugs against susceptible and 2 nd line treatment protocol against resistant *Mycobacterium tuberculosis*.
- Emphasizing over the research based rapid, reliable diagnostic test and vaccination for prevention and control of mycobacterial infection.
- Interventioning the health care professionals to reduce the probability to develop resistance to anti-TB drugs. That adversely influences the quality of health care outcomes. A well organized and deep rooted intervention can produce surprising benefits.
- Establishing effective monitoring of drugs used against tuberculosis in society.
- Conducting studies to update the professional knowledge improve the behaviors and inculcate the importance of completion of treatment course to control the tubercular infection in community and hospitals.

- Increasing the awareness of complications and threats posed by resistant *Mycobacterium tuberculosis* in community.
- Emphasizing to identifying the potential *Mycobacterium tuberculosis* strains accurately with molecular
- Ensuring crucially the understanding and participation in efforts to control the spread of resistance against anti-TB agents. Educational programmes describe the complications and provide guidelines for using the antituberculosis drugs in general practice and within institutional health care settings.

Recommendations for Prudential Treatment Practice

- Selecting carefully the treatment protocol and respective dose of anti-TB drugs. The medication decision is optimized on basis of pattern of sensitivity of patient's infected *Mycobacterium tuberculosis*.
- A high cure rate without the emergence of resistance can be achieved by adopting the treatments of internationally approved dose/ regimens. Which also are effective in prevention of the resistance emergence. Moreover; the combination chemotherapies minimize the probability of spontaneous mutant to develop resistance to all of the components of chemotherapy.
- Establishing the base line of effectiveness of antituberculosis agents, that is compare with: specifications founded in literature, data obtained from therapeutical & resistance surveillance studies and comparative data used to assess the international and/ or local risk factors.

Recommendations related to Epidemiology and resistance prevalence

- Restricting the over/ under compliance anti-TB drugs usage by offering the qualified pharmacist to play there professional role to develop the successful strategies. Pharmacist audit the prescriptions, evaluate the adherence to pharmacological considerations and formulary constrains to stop mechanism of resistance development.
- Convincing the drugs experts (pharmacists) and clinicians that anti-tuberculosis drugs therapy should be more closely tailored to the patients requires scientific proof. Prescription practice demands a strong mycobacterial profile, careful laboratory support and confirmed molecular elucidations.
- Establishing the concurrent morphological, molecular and genetic profile to assess the relationship between *Mycobacterium tuberculosis* emerging in treatment premises and community in various environments.
- Studying systematically to map the prevalence of bacterial pattern of resistance.
- Standardizing the methods used for diagnosis and sensitivity evaluation with the reference quality tests, which should closely follow the documents published by World Health Organization.
- Interpreting the correct culture and sensitivity results.
- Getting immunization against tuberculosis by BCG vaccination and improving the socio-economic status.

Therapeutical and Mycobacterium resistance management

- Characterizing and identification of Mycobacterium tuberculosis strains at primary health care level to coordinate the TB patient with the genotyping of detected strain. It will help in anti-tubercular drugs usage, monitoring and intervention programs at institution level.
- Facilitating to prescribe and/ or decide the treatment protocols with respect of mycobacterial clinical elucidations and therapeutical outcomes of pharmacological studies with the aim of improving the rational selection of regimens.

REFERENCES

- Ashok Rattan, Kalia A., Ahmed N. (1998).** Multidrug resistant *Mycobacterium tuberculosis*: Molecular perspectives; Emerging infectious diseases. All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India. Vol. 4 No.2.
- Banaiee N, Bobadilla-del-Valle M, Bardarov S, Riska PF, Small PM, Ponce-de-Leon A. (2001).** Evaluation of luciferase reporter mycobacteriophage technology for detection, identification, and antibiotic susceptibility testing of *Mycobacterium tuberculosis* complex in Mexico. Abstracts and final program of the 2001 Keystone Symposia on Molecular and Cellular Aspects of Tuberculosis Research in the Post Genome Era (B1). Taos, New Mexico. Abstract **204**, p. 69.
- Belanger AE, Besra GS, Ford ME, Mikusova K, Belisle JT, Brennan PJ, Inamine JM. (1996).** The *embAB* genes of *Mycobacterium avium* encode an arabinosyl transferase involved in cell wall arabinan biosynthesis that is the target for the antimycobacterial drug ethambutol. Proc Natl Acad Sci USA **93**:11919-24.
- Bergler H, Wallner P, Ebeling A, Leitinger B, Fuchshlschler S, Aschauer H, (1994).** Protein EnvM is the NADH-dependent-enoyl-ACP-reductase (Fab1) of *Escherichia coli*. J Biol Chem; **269**:5493-6.
- Bodmer T, Zurcher G, Imboden P, Telenti A. (1995).** Mutation position and type of substitution in the β -subunit of the RNA polymerase influence in-vitro activity of rifamycins in rifampicin resistant *Mycobacterium tuberculosis*. J Antimicrob Chemother **35**:345-8.
- Cangelosi G., Brabant W., Britschgl T. (1996).** Detection of rifampin- and ciprofloxacin- resistance *Mycobacterium tuberculosis* by using species-specific assays for precursor rRNA. Antimicrob. Agents Chemother; **40**: 1790-95.
- Karin C. Weyer, Bateman E., Blumberg L., Cameron N., Calver A., Churchyard G., Fourie B., Brown BG., Matji R. and Wilcox P. (1997).** The management of multidrug resistant tuberculosis in South Africa. Guidelines for the management of multidrug-resistant tuberculosis patients in South Africa. National Tuberculosis Research Programme, Medical Research Council, Pretoria.
- Cohn D., Bustreo F., and Raviglione M. (1997).** Drug-Resistant Tuberculosis: Review of the Worldwide Situation and the WHO/IUATLD Global Surveillance Project. Clinical Infectious Diseases, **24** (Suppl 1): S121-30
- Cooksey RC, Morlock GP, Glickman S, Crawford JT. (1997).** Evaluation of a line probe assay kit for characterization of *rpoB* mutations in rifampin resistant *Mycobacterium tuberculosis* isolates from New York City. J Clin Microbiol; **35**:1281-3.
- Dooley SW, Jarvis WR, Martone WJ, Snyder DE Jr. (1992).** Multi-Drug resistant tuberculosis [editorial]. Ann Intern Med 1992; **117**:257-8. El-Hajj, H., S. A. E. Marras, S. Tyagi, F. R. Kramer, and D. Alland. 2001. Detection of rifampin resistance in *Mycobacterium tuberculosis* in a single tube with molecular beacons. J. Clin. Microbiol. **39**:4131–37.

Edlin BR, Tokers JI, Greeko OV, Ovchin MH, Crawford JT, Williams J, Sordillo EM. (1992). An outbreak of multi-drug resistant tuberculosis among hospitalized patients with the Acquired Immuno-Deficiency syndrome. *N Engl J Med*; **326**:1514-21.

Felmlee TA, Liu Q, Whelen AC, Williams D, Sommer SS, Persing DH. (1995). Genotypic detection of *Mycobacterium tuberculosis* rifampin resistance: comparison of single strand conformation polymorphism and dideoxy fingerprinting. *J Clin Microbiol*; **33**:1617-23.

Garcia-Garcia ML, Jimenez-Corona ME, Ponce-de-Leon A, Jimenez-Corona A, Palacios-Martinez M, Balandrano-Campos S. (2000). *Mycobacterium tuberculosis* drug resistance in a suburban community in southern Mexico. *Int J Tuberc Lung Dis*; **4** (Suppl 2):168-70.

Granich RM, Balandrano S, Santaella AJ, Binkin NJ, Castro KG, Marquez-Fiol A. (2000). Survey of drug resistance of *Mycobacterium tuberculosis* in 3 Mexican states, 1997. *Arch Intern Med*; **160**:639-44

Heep M, Brandstatter B, Rieger U, Lehn N, Richter E, Rusch-Gerdes S. (2001) Frequency of *rpoB* mutations inside and outside the cluster I region in rifampin-resistant clinical *Mycobacterium tuberculosis* isolates. *J Clin Microbiol*; **39**:107–10.

Heifets LB, Lindholm-Levy PJ. (1992). Pyrazinamide sterilizing activity in vitro against semidormant *Mycobacterium tuberculosis* populations. *American Review of Respiratory Diseases*; **145**:1223-5.

Hirano K, Abe C, Takahashi M. (1999). Mutations in the *rpoB* gene of rifampin-resistant *Mycobacterium tuberculosis* strains isolated mostly in Asian countries and their rapid detection by line probe assay. *J Clin Microbiol*; **37**:2663-66.

Herendra Thakker and JR Sah. (1998). Multidrug resistance pulmonary tuberculosis. *Ind. J. Tub.*, **45**, 131

Heym B, Alzavi PM, Honore N, Cole ST. (1995). Missense mutations in the catalase-peroxidase gene, *katG*, are associated with isoniazid resistance in *Mycobacterium tuberculosis*. *Mol Microbiol*; **15**:235-45.

Hoffner SE, Klintz L, Olsson-Liljequist B, Bolmstrom A. (1994). Evaluation of E test for rapid susceptibility testing of *Mycobacterium chelonae* and *M. fortuitum*. *J Clin Microbiol*; **32** : 1846-9.

Iseman MD, Sbarbaro JA. (1992). The increasing prevalence of resistance to antituberculosis chemotherapeutic agents: implications for global tuberculosis control. *Curr Clin Top Infect Dis*; **12**:188-204.

Jaber M, Rattan A, Kumar R. (1996). Presence of *katG* gene in isoniazid-resistant strains of *Mycobacterium tuberculosis*. *J Clin Pathol*; **49**:945-7.

Johnsson K, King DS, Schultz PG. (1995). Studies on the mechanism of action of isoniazid and ethionamide in chemotherapy of tuberculosis. *Journal of American Chemical Society*; **117**:5009-10.

Julio C. Mendez. (1997). Multi Drug Resistance in Tuberculosis and the Use of PCR for Defining Molecular Markers of Resistance. Dept. of Medicine, University of Florida and Duval County Health Department, Jacksonville, Florida

Kakkar N, Sharma M, Ray P, Sethi S, Kumar S. (2000). Evaluation of E test for susceptibility testing of *Mycobacterium tuberculosis* to primary anti-tubercular drugs. *Indian J Med Res*; **111** : 168-71.

Kalia A, Ahmad N, Rattan A. (1997). Diagnosis of multi-drug resistant tuberculosis: comparison of traditional, radiometric and molecular methods [abstract]. In: Abstracts of the 20th International Congress of Chemotherapy; 29 Jun-3 Jul 1997; Sydney, Australia. Sydney: International Society of Chemotherapy;. p. 211.

Kapur V, Li LL, Iordanescu S, Hamrick MR, Wanger A, Kreisworth RN. (1994). Characterization by automated DNA sequencing of mutations in the gene (*rpoB*) encoding the RNA polymerase β -subunit in rifampicin-resistant *Mycobacterium tuberculosis* strains from New York City and Texas. *J Clin Microbiol*; **32**:1095-8.

Kato-Maeda M, Sifuentes-Osornio J, Bobadilla-del-Valle M, Ruiz-Palacios GM, Ponce-de-Leon A. (1999). Drug resistance among acid-fast bacilli. *Lancet*; **353**:1709.

Kim BJ, Kim SY, Park B-H, Liu M-A, Park I-K, Bai GH. (1997). Mutations in the *rpoB* gene in *Mycobacterium tuberculosis* that interfere with PCR-single strand conformation polymorphism analysis for rifampin susceptibility testing. *J Clin Microbiol*; **35**:492-4.

Edward K.J., Metherell L.A., Yates M. and Saunders N.A. (2001). Detection of *rpoB* mutation in *Mycobacterium tuberculosis* by biprobe analysis. *J. of Clinical Microbiology*, September, p 3350-3352, Vol. **39**, No.9

Kochi A, Vareldzis B, Styblo K. (1993). Multi-Drug resistant tuberculosis and control. *Res Microbiol*; **144**:104-10

Maggliozzo RS, Marcinkeviciene JA. (1996). Evidence for isoniazid oxidation by oxypressors mycobacterial catalase-oxidase. *Journal American Chemical Society*; **118**:11 303-4.

Masur H. (1993). Recommendations on prophylaxis and therapy for disseminated *Mycobacterium avium* complex disease in patients infected with HIV virus. *N Engl J Med*; **329**:828-33.

Mdluli K, Sherman DR, Hickey MJ, Kreiswirth BN, Morris S, Stover CK, Barry LE III. (1996). Biochemical and genetic data suggest that *inhA* is not the primary target for activated isoniazid in *Mycobacterium tuberculosis*. *J Infect Dis*; **174**:1085-90.

Miller LP, Crawford JT, Shinnick TM. (1994). The *rpoB* gene of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother.*; **38**:805–11.

Miriam Bobadilla-del-Valle, Leon APC., Huertero CA., Alarcon GV., Maeda MK., Small M., Coury P., palacios R and Osornio JS. (2001). *rpo B* Mutations in rifampicin resistant *Mycobacterium tuberculosis* identified by polymerase chain reactions single strand conformational polymorphism. Stanford University Medical Centre, Stanford, California, USA. Vol. 7, No. 6, Mitchison DA. 1985. Mechanism of drug action in short-course chemotherapy. *Bulletin International Union Against Tuberculosis*; **65**:30-7.

Mitchison DA., Nuna AJ. (1986). Influence of initial drug resistance on the response to short-course chemotherapy of pulmonary tuberculosis. *Am Rev Respir Dis*; **133**:423-30.

Mokrousov, I., O. Narvskaya, T. Otten, A. Vyazovaya, E. Limeschenko, L., Steklova, and B. Vyshnevskiy. (2002). Phylogenetic reconstruction within *Mycobacterium tuberculosis* Beijing genotype in northwestern Russia. *Res. Microbiol.***153**:629–37.

Jain N.K. (1996). Laboratory diagnosis of pulmonary tuberculosis: conventional and newer approaches. *Ind. J. Tub.* **196**, 43, 107

Nagwa Khamis, Amin MN., Zagloul MZ., Girgis SA.and Shetta M. (2004). DNA sequencing and bacteriophage based technique for rapid detection of rifampicin resistant *Mycobacterium tuberculosis*. *Egypt J. Medical Lab. Sci., (ESIC)*, Vol. **13**, No.1,

Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T. (1989). Detection of polymorphisms of human DNA by gel electrophoresis single-strand conformation polymorphisms. *Proc Natl Acad Sci U S A*;**86**:2766-70.

Ovchinnikov YA, Monastyrskaya, GS, Gubanov VV, Lipkin VM, Sverdlov ED, Kiver IF. (1981). The primary structure of *Escherichia coli* RNA polymerase. Nucleotide sequence of *rpoB* gene and amino-acid sequence of the β -subunit. *Eur J Biochem*;**116**:621-9.

Pearson ML, Jareb JA, Freiden TR, Crawford JT, Davis BJ, Dooley SN. (1992). Nosocomial transmission of multi-drug resistant tuberculosis—a risk to patients and health care workers. *Ann Intern Med*;**117**:191-6.

Pretorius GS, Van Helden PD, Sergel F, Eisenach KD, Victor TC. (1995). Mutations in *katG* gene sequences in isoniazid-resistant clinical isolates of *Mycobacterium tuberculosis* are rare. *Antimicrob Agents Chemother*;**39**:2276-81.

Quemard A, Sacchetti JC, Dessen A, Jacobs WR Jr, Blanchard JS. (1995). Enzymatic characterization of the target for isoniazid in *Mycobacterium tuberculosis*. *Biochemistry*;**34**:8235-41.

Raviglione M.C., Smidar D.E. Kochin A. (1995). Global epidemiology of tuberculosis. morbidity and mortality of a world wide epidemic. *JAMA*, **273**,220-26.

Richard D. Howland, Mycek J.M., Harvey R.A., Champe P.C. (2006). Lippincott's Illustrated reviews: Pharmacology. 3rd Edition (2006). 351 West Camden Street Baltimore,MD **21201**. p 395- 40

Sachdeva R, Gadre DV, Talwar V. (2002). Characterization and drug susceptibility patterns of extrapulmonary mycobacterial isolates. *Indian J Med Res*; **115** : 102-07.

Sherman DR, Sabo PJ, Hickey MJ, Arain TM, Mahairas GG, Yuan Y. (1995). Disparate responses to oxidative stress in saprophytic and pathogenic mycobacteria. *Proc Natl Acad Sci U S A*;**92**:6625-9.

Sifuentes-osornio J, Ponce-de-leon A, Camacho-Mezquia FE., Pobadilla S. Joy Milan, Kirsten A. Hauge, Natalia E. Kurepina. (2004). Expanded Geographical Distribution of the N Family of *Mycobacterium tuberculosis* Strains within the United States. *Seattle; Journal of Clinical Microbiology*, , p. 1064–1068 Vol. **42**, No. 3

Snider DE Jr, Roper WL. (1992). The new tuberculosis. *N Engl J Med*;**326**:703-5.

Sonal S. Munsiff, Bassoff T, Nivin B., Li J., Sharma A., Mathema B., Driscoll J., and Kreiswirth BN. (1997). Molecular Epidemiology of Multidrug-Resistant Tuberculosis, New York City,. Tuberculosis Genotyping Network. New York. USA

Sreevatsan S, Pan X, Zhang Y, Deretic V, Muser JM. (1997). Analysis of the *oxyR-ahpC* region in isoniazid-resistant and -susceptible *Mycobacterium tuberculosis* complex organisms recovered from diseased humans and animals in diverse localities. *Antimicrob Agents Chemother*; **41**:600-6.

Telenti, A., P. Imboden, F. Marchesi, D. Lowrie, S. Cole, M. J. Colston, L. Matter, K. Schopfer, and T. Bodmer. (1993). Detection of rifampicin resistance mutations in *Mycobacterium tuberculosis*. *Lancet* **341**:647–50

Takayama K, Kilburn JO. (1989). Inhibition of synthesis of arabinogalactan by ethambutol in *Mycobacterium smegmatis*. *Antimicrob Agents Chemother*; **33**:1493-99.

Van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenbach KD, Gicquel P, (1993). Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. *J Clin Microbiol* **31**:406–09.

Victor TC, Warren R, Butt JL, Jordaan AM, Felix JV, Venter A, Sirgel FA, Schaaf HS, Donald PR, Richardson M, Cynamon MH, Van Helden. (1997). Genome and MIC stability in *Mycobacterium tuberculosis* and indications for continuation of use of isoniazid in multidrug-resistant tuberculosis. 1: *J Med Microbiol.* **46(10)**:847-57

Vitali Sintchenko, Jelfs PJ., Chew W.K. and Gilbert GL. (1999). *Mycobacterium tuberculosis* rpo B gene DNA sequencing: implications for detection of Rifamycin resistance. *J anti-Microb Chemother* **44**: 294-95.

Whelen AC, Felmler TA, Hunt JM, Williams DL, Roberts GD, Stockman L, Persing DH. (1995). Direct genotype detection of *Mycobacterium tuberculosis* rifampicin resistance in clinical specimens by using single-tube heminested PCR. *J Clin Microbiol* **33**:556-61.

Williams DL, Waguespack C, Eisenach K, Crawford JT, Portaels M, Salfinger M, (1994). Characterization of rifampicin-resistance in pathogenic mycobacteria. *Antimicrob Agents Chemother* **38**:2380-86.

Wilson TM, Collins DM. (1996). *ahpC*, a gene involved in isoniazid resistance of *Mycobacterium tuberculosis* complex. *Mol Microbiol* **19**:1025-34.

WHO. (2004). World Health Organization Surveillance of drug resistant tuberculosis in South East Asia. Report of an Inter-country training course Bangalore, India, 21, SEA-HLM-383 SEA-TB-270.

Zhang Y, Garbe T, Young D. (1993). Transformation with *katG* restores isoniazid-sensitivity in *Mycobacterium tuberculosis* isolates resistant to a range of drug concentrations. *Mol Microbiol* **8**:521-24.